Original Article

Frequency of Class 1 Integron and Genetic Diversity of *Acinetobacter baumannii* Isolated from Medical Centers in Kermanshah

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Abstract

Background and Objective: *Acinetobacter baumannii* has emerged as an important pathogen in hospital and environment that can acquire transport element and antibiotic-resistant genes. The aim of this study was to determine the resistances to different antibiotics, frequency of Class 1 integron in *A. baumannii* and then molecular typing for *A. baumannii* isolated from Intensive Care Unit (ICU). **Materials and Methods:** A total of 100 isolates of *A. baumannii* were collected from patients admitted to hospitals in Kermanshah from April 2014 to September 2015. The isolates were identified using biochemical test. Antimicrobial susceptibility test for 20 antibiotics was determined by Kirby–Bauer antibiotic testing (or disc diffusion). The prevalence rate of class integrons among the isolates was determined using polymerase chain reaction and finally 80 isolates of *A. baumannii* obtained from the Intensive Care Unit were selected for molecular typing by pulsed-field gel electrophoresis (PFGE). **Results:** The maximum drug resistance was observed against cefotaxime, ceftriaxone, mezlocillin, imipenem, and ceftazidime and piperacillin. Twenty-nine isolates were multidrug resistant (MDR); about 21 isolates were extensively-drug resistant and none were pandrug resistance and 42 isolates (42%) contained Class 1 integrons. The results did not show a significant correlation between the presence of Class 1 integrons and incidence of MDR *A. baumannii*. Five clusters were obtained by PFGE. **Conclusion:** This study did not show a significant correlation between the presence of Class 1 integrons and incidence of MDR *A. baumannii*. By PFGE analysis, the high level of similarity between some pulsotypes in *A.baumannii* strains showed genetic correlation between them.

Keywords: *Acinetobacter baumannii*, Class 1 integron, pulsed-field gel electrophoresis

Introduction

Acinetobacter baumannii is a coccobacilli, Gram-negative, nonmoving, oxidase – negative, *Bacteria*.[1] This human pathogenic bacterium is opportunistic, which will be causes spread hospital infection, including bacteremia, urinary, tract infection, and pneumonia.[2] This organism can be preserved in environment, condition and through pollution levels lead to the spread of infection in hospital.[3] The integrons in *A. baumannii* isolates, are a mobile genetic elements and they have ability to acquire and expand expressed of the antimicrobial resistance factors.[4] Integrons of Class 1 and 2 are the most recognized integrons that were frequently in *A. baumannii* and they had resistance with multiple categories to beta-lactams, aminoglycosides, fluoroquinolone and carbapenems.[5] The frequency of multidrug-resistant (MDR) *A. baumannii* isolates increased in last decade. MDR was defined resistant to three classes of antibiotics, including cephalosporins (cefepime and ceftazidime) carbapenems (imipenem and meropenem), and quimolones (ciprofloxacin) and also resistant to carbapenem, called extensively-drug resistant (XDR). Pandrug resistance (PDR) *A. baumannii* isolates shall be the XDR *A. baumannii* isolates that were resistant to tigecycline and polymyxins.[6] The aim of this

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study was to determine the resistances to different antibiotics, frequency of Class 1 integron in *A. baumannii* and then molecular typing for *A. baumannii* isolates that obtained from Intensive Care Unit (ICU) was carried out by pulsed-field gel electrophoresis (PFGE).

Materials and Methods

Bacterial isolates

A total of 100 clinic isolates of *A. baumannii* were collected from patients admitted in Kermanshah during April 2014–September 2015 from four hospitals, which includes Taleghani (A), Imam Reza (B), Imam Khomeini (C), and Mohammad Kermanshahi (D). These isolates obtained from sputum, blood, urine, wounds, abdominal abscesses, synovial were identified using biochemical tests such as oxidase test, and environment TSI, API 20 NE kit and to ensure that these samples are *A. baumannii* use of growth in 42°C.

Antimicrobial susceptibility test

This isolates sensitivity to twenty antibiotics from most company that include: Ceftriaxone (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), meropenem (10 μg), cefepime (30 μg), amikacin (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), polymixin B (300 units), gentamicin (10 μg), imipenem (10 μg), mezlocycline (15 μg), minocycline (30 μg), tetracycline (30 μg), tigecycline (15 μg), colistin (10 μg), tobramycin (10 μg), gatifloxacin (5 μg), piperacillin (100 μg), co-trimoxazole (1.25 μg) using table of CLSI 2012, and disk diffusion determines their sensitivities.

Polymerase chain reaction assay

DNA genomic of all *A. baumannii* isolates were extracted by boiling method (5–10 min in 100°C). Detection of Class 1 integron in clinical isolates of *A. baumannii* was carried out by polymerase chain reaction (PCR) with primers illustrated in Table 1 as described previously by Mirnejad *et al*.[7]

Primers Class 1 integron/F/R were used to amplify 160 bp fragments [Table 1], according to the following program: Initial denaturation at 95°C for 5 min, followed by 30 cycles at 49°C for 30 s, 55°C s 72°C for 3 s, and a final extension at 72°C for 5 min. All PCR amplification was performed in duplicate. The PCR product were analyzed using electrophoresis technique an 0.26 g/L agarose gel for 40 s at 80 VOH, strained by ethidium bromide, and visualized under transilluminator. Amplification products were further evaluated by sequencing.

Table 1: Primers of polymerase chain reaction

Primers	Nucleotide sequence	Expected amplification size (bp)
5′-cs	GGCATCCAAGCAGCAAG	Variable
3′-cs	AAGCAGACTTGACCTGA	Variable
Int1-F	CAGTGGACATAAGCCTGTTC	160
Int1-R	CCCGAGGCATAGGCTGTA	160

Statistical analysis

Statistical analyses were performed using SPSS (version 20) (IBM, Chicago, IL, USA). Fisher's and Chi-square exact tests were used to the relationship between antibiotic resistant pattern and integron-positive genotype. $P < 0.05$ was considered statistically significant.

Pulsed-field gel electrophoresis

PFGE analysis was performed using methods (cell lysis, cell washing, and digestion by *ApaI* restriction enzyme) as described Mohajeri *et al.* without any change.[8]

Results

A total of 100 *A. baumannii* isolates were highly resistant to ceftriaxone, cefotaxime, mezlocillin (>90%) and imipenem, ceftazidime, piperacillin (>80%) [Tables 2 and 3] and were susceptible to colistin, polymyxin B, minocycline and tetracycline. MDR Bactria were mainly isolated from sputum (60%), abdominal abscesses (19%), blood (6%), urine (5%), wound (2%), synovial fluid (2%), from male (61%), age (mean: 40.3 ± 25.1, minimum = 1, maximum = 83), and female (39%) age (mean: 40.3 ± 25.7, minimum = 1, maximum = 83). MDR and XDR frequencies were 29% and 21%, respectively, and none were PDR. The results were analyzed by Chi-square Fisher (KI2) using SPSS software and $P = 0.05$. According to our result, there was not related to the incidence of MDR ($P = 0.2$) and XDR ($P = 0.5$) with Class 1 integron. 5′-cs-3′-cs was the area with variable region and there were the genes cassette intgrates and performed for detection of complete Class 1 (42, 76%) [Figure 1]. According to the [Table 4], resistance to cefepime, tobramycin, polymyxin B and ceftazidime, with Class 1 integron was significant. Results of this study showed that resistance to Levofloxacin, cefepime, mezlocillin, ciprofloxacin, piperacillin, cefotaxime, ceftazidime, ceftriaxone, and gentamicin are high in ICU ward [Table 3]. Five clusters were obtained by PFGE including: A ($n = 23$), B ($n = 21$), C ($n = 18$), D ($n = 17$) and E ($n = 1$) [Figure 2]. Eighty (80%) strains were isolated from Hospital A, B and C. All were isolated from ICU and the most from respiratory tract secretions sample. Clone A was dominant and widespread in ICU ward Taleghani Hospital, other clones especially Clone B were in the next dominant clones after Clone A. Also Clone A and B (16%) had the most frequency in the male. Pulsotype E consisted of single isolate and isolated from the female patient. All isolates belonging to the same genotype had similar genotype and phenotypes, such as distribution gene (Class 1 integron), resistance pattern, and source of isolation [Table 5].

Discussion

Over the last decade, *Acinetobacter* has emerged to become an important cause of nosocomial infections in many parts of the world.[8,9] *A. baumannii* is typically resistant to various antimicrobial agents such as penicillins,

Table 2: Resistance in different antibiotic classes

Antibiotic class	Tested members	Susceptible	Intermediate	Resistant
Fluoroquinolones	Levofloxacin	21	7	72
	Gatifloxacin	38	14	48
	Ciprofloxacin	19	0	81
Aminoglycoside	Tobramycin	37	1	62
	Gentamicin	17	2	81
	Tigecycline	93	1	6
	Amikacin	18	7	75
Carbapenem	Meropenem	18	8	74
	Imipenem	15	3	82
Spread range of penicillin	Piperacillin	12	6	82
	Mezlocillin	8	1	91
Sulfonamide	Cotrimoxazole	26	0	74
	Polymixin B	82	11	7
	Colistin	72	16	12
Tetracycline	Tetracycline	22	6	72
	Minocycline	77	13	10
Cephalosporines	Cefepime	11	4	85
	Cefotaxime	7	0	93
	Ceftazidime	13	1	86
	Ceftriaxone	2	6	92

Table 3: The table of the resistance of antibiotics

Different antibiotics	Wards				*P*
	ICU (%)		Other ward (%)		
	Resistant	Susceptible	Resistant	Susceptible	
Cefepime	19 (70.4)	8 (29.6)	66 (90.4)	7 (9.6)	0.013*
Tigecycline	12 (3.7)	26 (96.3)	5 (6.8)	68 (93.2)	0.557
Tobramycin	16 (59.3)	11 (40.7)	46 (63.0)	27 (37.0)	0.731
Polymixin B	2 (7.4)	25 (92.6)	5 (6.8)	68 (93.2)	0.923
Mezlocillin	20 (74.1)	7 (25.9)	71 (97.3)	2 (2.7)	0.001*
Levofloxacin	15 (55.6)	12 (44.4)	57 (78.1)	16 (21.9)	0.026*
Cefotaxime	25 (92.6)	2 (7.4)	68 (93.2)	5 (6.8)	0.923
Minocycline	11 (3.7)	26 (96.3)	9 (12.3)	64 (87.7)	0.202
Gatifloxacin	9 (33.3)	18 (66.7)	39 (53.4)	34 (46.6)	0.074
Colistin	1 (3.7)	26 (96.3)	11 (15.1)	62 (84.9)	0.121
Tetracycline	12 (44.4)	15 (55.6)	60 (82.2)	13 (17.8)	0.001*
Meropenem	18 (66.7)	9 (33.3)	56 (76.7)	17 (23.3)	0.309
Ciprofloxacin	14 (51.9)	13 (48.1)	67 (91.8)	6 (8.2)	0.001*
Piperacillin	16 (59.3)	11 (40.7)	66 (90.4)	7 (9.6)	0.001*
Cotrimoxazole	15 (55.6)	12 (44.4)	59 (80.8)	14 (19.2)	0.011*
Ceftazidime	17 (63.0)	10 (37.0)	69 (94.5)	4 (5.5)	0.001*
Ceftriaxone	22 (81.5)	5 (18.5)	70 (95.9)	3 (4.1)	0.018*
Amikacin	17 (63.0)	10 (37.0)	58 (79.5)	15 (20.5)	0.091
Gentamicin	16 (59.3)	11 (40.7)	65 (89.0)	8 (11.0)	0.001*
Imipenem	21 (77.8)	6 (22.2)	61 (83.6)	12 (16.4)	0.504

*: Significant

cephalosporines, macrolides, aminoglycosides, tetracyclines and fluoroquinolones.[10] Multidrug-resistant *Acinetobacter* spp. is alert pathogens, mostly in ICUs and is related with outbreaks of infection, which require epidemiologic monitoring as a measure for controlling nosocomial infection.[11] In this study, the majority of *Acinetobacter* spp. was isolated from the ICU. These results are in consistent with that reported by Al Masoudi *et al*.[9] in our study showed that the highest number of isolates related to sputum 66%, similar to with other studies.[12,13] In this study we studied susceptibility pattern of the 100 clinical isolates of *A. baumannii* to 20 different antibiotics as common therapeutic drugs in hospitalized patients. Resistance ratios

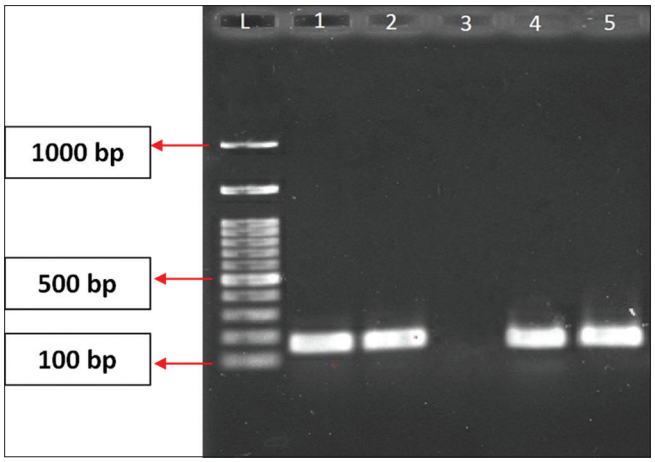

Figure 1: Polymerase chain reaction image of Class 1 integron. L = Ladder 100 bp, 1, 2 and 5 = sample, 3 = negative control, 4 = positive control

each antimicrobial agent was all above 50%. The susceptibility of clinical isolates to tigecycline (93%), polymyxin B (82%), minocycline (77%), colistin (72%) is move that 70%. Only these four antimicrobial agents had a tolerable killing ability of clinical *A. baumannii* from this hospital. Therefore, treatments of these infections are complicated. The resistance of the *A. baumannii* isolates showed that 29% were MDR and twenty-one (21%) isolates were XDR. The frequency of MDR isolates was reported by Mohajeri *et al*.[8] in 2015 at Kermanshah, Iran, were 93% among *A. baumannii* isolates, the same as this study. The prevalence of MDR and XDR causes epidemic in different geographic area. The inclusion of MDR is related to other genetic factors. It seems resistance was through the different ways such as deficiency in the enzyme, in the cell wall, or via plasmid and chromosome that in our study did not include. Most of the MDR isolates belonged to patients in ICU which admits patients. In the ICU indiscriminate use of antimicrobial agents, long hospital stay, prolonged use of catheters and ventilators, implants and other synthetic instruments, lead to pressure select resistant strains which were colonized in susceptible patients.[14]

Class 1 integron being the most common among the clinical isolates of Gram-negative *Bacteria*, including acinetobacters.[10] The results related to Class 1 integron were in accordance with other geographical regains including European countries (43%),[15] and China (51.9%).[16] Horizontal gene transfer plays a significant role in cases increase the resistant antibiotics with Class 1 integron and publishing antibiotics gene in Class 1 integron. In the Mirnejad *et al*.'s study, 42% of MDR isolates contained Class 1 integron,[7] the same as this study. In a study in 2009 on 97 *A. baumannii* in the United States, 80% of isolates were MDR,[17] in contrast with our study. In the present study, there was a significant association between resistance to tobramycin, cefepime, ceftazidime and polymyxin B with integron carriage. No significant association was found between presence of integrons and resistance to antibiotics. These results suggest that resistance to antibiotics is independent of resistance gene

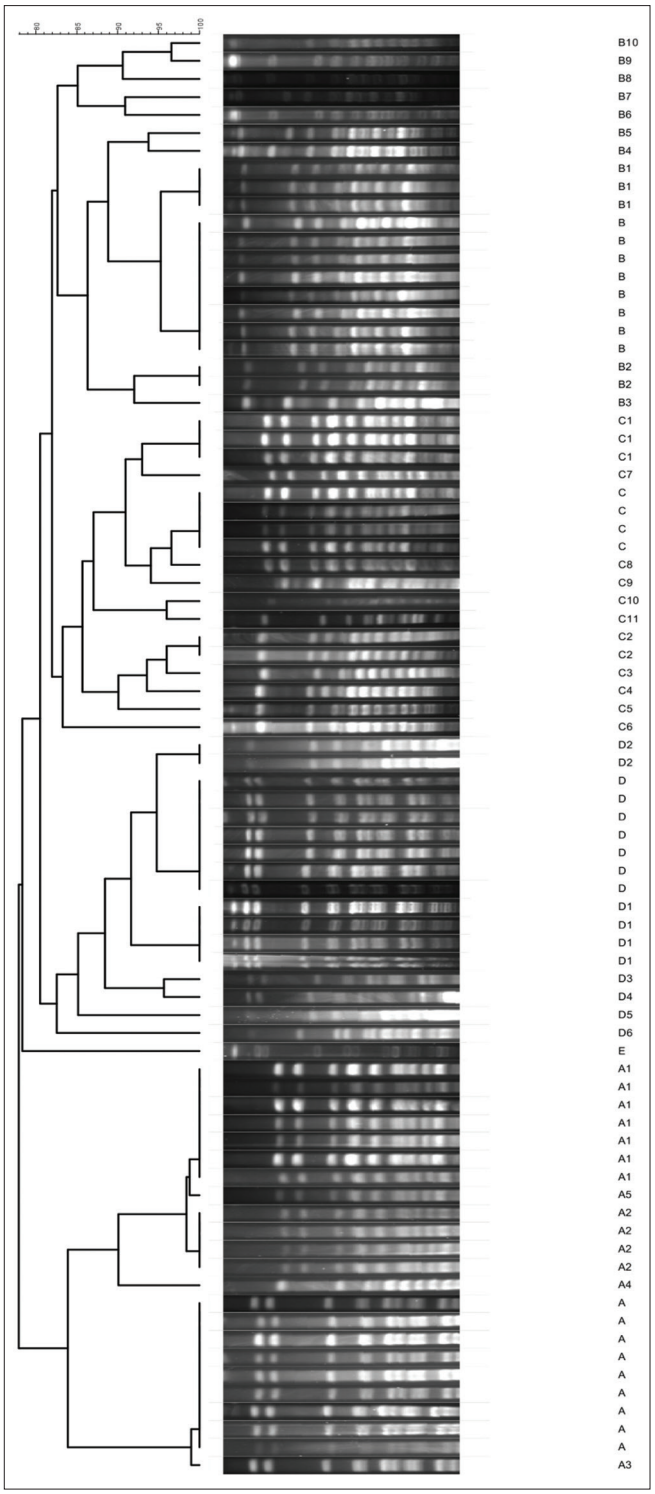

Figure 2: Pulsed-field gel electrophoresis dendrogram of *Acinetobacter baumannii* isolates

cassettes carried by integron Class 1. It seems resistance was through the different ways such as deficiency in the enzyme, in the cell wall, or vial plasmid and chromosome that in our study did not include, which is compatible with studies done by Japoni *et al*.[18] PFGE is the gold standard technique to investigate the molecular epidemiology of *Bacteria*.[19] By

Table 4: The table of relation between ward and Class 1 integron

Different antibiotics	Class 1 integron		*P*
	Resistant	Susceptible	
Cefepime	40 (95.2)	2 (4.8)	0.015*
Tigecycline	4 (9.5)	38 (90.5)	0.207
Tobramycin	31 (73.8)	11 (26.2)	0.038*
Polymixin B	0 (0.0)	42 (100)	0.020*
Mezlocillin	40 (95.2)	2 (4.8)	0.208
Levofloxacin	30 (71.4)	12 (28.6)	0.914
Cefotaxime	37 (88.1)	5 (11.9)	0.102
Minocycline	3 (7.1)	39 (92.9)	0.418
Gatifloxacin	20 (47.6)	22 (52.4)	0.774
Colistin	5 (11.9)	37 (88.1)	0.980
Tetracycline	33 (78.6)	9 (21.4)	0.213
Meropenem	31 (73.8)	11 (26.2)	0.971
Ciprofloxacin	36 (85.7)	6 (14.3)	0.306
Piperacillin	35 (83.3)	7 (16.7)	0.768
Cotrimoxazole	33 (78.6)	9 (21.4)	0.375
Ceftazidime	40 (95.2)	2 (4.8)	0.023*
Ceftriaxone	38 (90.5)	4 (9.5)	0.633
Amikacin	35 (83.3)	7 (16.7)	0.101
Gentamicin	37 (88.1)	5 (11.9)	0.124
Imipenem	35 (83.3)	7 (16.7)	0.768

*: Significance

Table 5: Distribution of pulsed-field gel electrophoresis pattern, anti-microbial susceptibility and Class 1 integron of *Acinetobacter baumannii* isolates

Clone PFGE	Number	MDR	XDR	Int1
Clone A	23	8	6	10
Clone B	21	10	9	20
Clone C	18	6	4	7
Clone D	17	4	2	4
Clone E	1	1	0	1
Total	80	29	21	42

PFGE: Pulsed-field gel electrophoresis, MDR: Multidrug resistant, XDR: Extensively drug resistant

PFGE method, we obtained 5 clones that the Clone A was involved in the majority of outbreaks in Kermanshah. It occurred at ICU and Isolates within this clone were mainly positive for Class 1 integron (%83). Clone B was the second most common pattern involved in outbreaks. Most of the *A. baumannii* infections were caused by a few predominant clones. Most of MDR and XDR phenotypes were presented in the Clones A, B, and C. It is possible that the transfer of these clones to other wards by staff or hospital equipments. A high prevalence of the Clone A, B, and C in different parts of the health-care system showed that hospitalized patients should be highly careful to prevent the spread of these clones. As expected, there was no significant relation among strains in term of correlation between Class 1 integron and MDR. This indicates that this gene could be role in common of the *A. baumannii* in ICU ward. More investigations are required to find putative source of wide distribution of Class 1 integrons. It is difficult to prove whether patients were colonized or infected by *A. baumannii* especially, where there are not previous epidemiologically records.

Conclusion

This study showed that resistance is also more pronounced in the ICU. Infections of ICU are serious problems that can compromise patient's survival and the outcome of reconstructive treatment. Since did not detect any association between resistances to different classes of antibiotics with integrons. Monitoring of drug resistance with use of coexist gene integrase PCR and other IS element are seems very important to plan specific infection control measures. PFGE analysis is helpful for the detection of common strains in different wards and prevention of further spread of these pulsotypes to other hospital environment. Different patterns of PFGE among hospitalized show wide distribution of integrons which reflect the issue in the case of endemic. The high level of similarity between some pulsotypes in *A. baumannii* strains showed genetic correlation between them. Further investigations are required for analyzing other PCR bands on a wider range of bacterial collection to detect other integrated gene cassettes.

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Conflicts of interest

There are no conflicts of interest.

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