

Prevalence of Class 2 Integrons in Multidrug-Resistant *Acinetobacter Baumannii* in Toxicological ICU Patients in Tehran

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ABSTRACT

Background: *Acinetobacter baumannii* is an important opportunistic pathogen which causes complications in hospitalized patients, especially those in ICU. The aim of this study was to determine the frequency of class 1 and 2 integrons in multi-drug resistance *A. baumannii* and to investigate the association between the presence of integrons and antibiotic resistance patterns.

Methods: A total of 40 *A. baumannii* strains were isolated from 372 ICU patients from June to Oct 2012. *A. baumannii* was detected in 50% of tracheal cultures, 15% in blood, 15% in urine samples, and 22.5% in other locations. In accordance with CLSI 2011, 12 antibiotics were used through disc diffusion method. Existence of integron classes was investigated by PCR assay with the amplification of integrase genes.

Results: The most effective antibiotic against *Acinetobacter baumannii* was polymyxin B with 100% susceptibility, followed by meropenem, piperacillin, cotrimoxazole, ceftazidime with 100% resistance; this was followed by ciprofloxacin 97.5%, tetracycline, 92.5%, imipenem 62.5%, and gentamicin 60% resistance. The presence of integron class 1 was 7.5%, class 2 was 67.5%, and non-integron was 20%.

Conclusion: The association between multidrug resistance and class 2 integron was not statistically significant. Other factors accounting for the lack of significance of the findings may be the impact of other resistance determinants such as transposons or plasmids, not investigated in the current study. Considering the increasing trend of MDR infections among ICU patients with critical problems in follow up, the use of appropriate infection control strategy and a regular surveillance system is necessary in our hospital.

Keywords: *Acinetobacter Baumannii*, Class 1 Integron, Class 2 Integron, Multidrug Resistance, PCR Assay.

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INTRODUCTION

Acinetobacter baumannii (*A. baumannii*) is an important opportunistic pathogen which causes complications in hospitalized patients, especially those in intensive care units (ICU) due to the acquisition of multidrug resistance (MDR) [1]. Numerous recent global outbreaks have been attributed to *A. baumannii*, an important motivation for this study. Existence of antibiotic resistance genes located on integrons among acinetobacter spp has been reported in other studies [1-3]. Integrons are

DNA elements capable of capturing genes by a site-specific recombination mechanism that often carry gene cassettes, containing antibiotic resistance genes [1-3].

Several classes of integrons have been described, with class 1 integrons being the most common and widely distributed among gram-negative bacteria [1]. The second class of integrons is found in transposon [4]. Recently, the incidence of integron class 2 has increased [5]. There are increasing reports of multidrug resistant *A. baumannii* (MDRAB) outbreaks in clinical settings worldwide [5-7].

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A significant increase in the prevalence of MDR gram-negative bacilli was reported [8-10]. Carbapenems were the most successful β -lactam antibiotics in evading bacterial resistance [9-11]. Recently, carbapenems resistance, mediated by acquired carbapenemase genes, has been increasingly reported particularly for clinical isolates of *Pseudomonas aeruginosa* and *A. baumannii* spp. [7, 12, 13]. *A. baumannii* is resistant to piperacillin, cefepime, ceftriaxone, 100%, 99% and 97%, respectively [1] while only colistin was 100% sensitive in most of the studies [4]. Also, *A. baumannii* is resistant to the aminoglycosides amikacin 80% and gentamicin 85% [7]. Most integron positive isolates were resistant to aminoglycoside compounds, possibly through the existence of many gene cassettes conferring resistance to aminoglycosides [7, 14].

The objectives of this study were to determine the frequency of class 1 and 2 integrons among MDRA clinical isolates and to investigate the association between the presence of integrons and antibiotic resistance patterns in this microorganism.

MATERIALS AND METHODS

Patients and Setting

In an analytical cross-sectional study in a 5-month period, from June to October 2012, samples were collected from a total of 362 ICU inpatients in Loghman Hakim General Hospital, a poisoning patient referral center affiliated with Shahid Beheshti University of Medical Sciences (SBMU), situated in the south of Tehran, the capital city of Iran. The hospital includes General and Toxicology ICU units and in this study 172 patients selected from the General ICU and 190 patients from the Toxicology ICU. Overall, 62 patients were detected as clinically suspicious for *Acinetobacter* infections. All patients had been hospitalized for at least 48 hours. This study was approved by the Research Ethics Committee of SBMU (code no. 90-1-113-8454).

Isolation of *Acinetobacter*

Biologic samples included tracheal aspiration, urine, blood, and wound cultures. Repeated species from the same patient were

excluded. All isolates were identified using standard bacteriologic and biochemical methods, such as gram stain, oxidase test, motility, catalase test, citrate test, O/F (oxidation-fermentation) test and growth at 37°C and 44°C [12,6]. Overall, 40 *Acinetobacter* strains were isolated.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the standard Mast Co, UK disc diffusion method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines [6]. The antimicrobial agents tested were meropenem (10 μ g), gentamicin (10 μ g), tetracycline (30 μ g), imipenem (10 μ g), ciprofloxacin (5 μ g), cotrimoxazole (25 μ g), piperacillin (100 μ g), piperacillin tazobactam (100/10 μ g), ceftazidime (10 μ g), polymyxinB (300 μ g), ceftriaxone (30 μ g), cefepime (30 μ g), and cefotaxime (30 μ g). *Pseudomonas aeruginosa* ATCC 27853 was used as the control strain in antimicrobial susceptibility testing.

Dna Extraction and Pcr Assay

Extraction of genomic DNA from *A. baumannii* isolates was performed by iNtRON kit. Determination of integron classes 1 and 2 was performed by conventional PCR using the primers BioNeer (Table 1).

Table 1. Primers used in the study.

Primer	Nucleotide Sequence	Reference
Int1-F	5' CAG TGG ACA TAA GCC TGT TC 3'	Koeleman et al.,2000
Int1-R	5' CCC GAG GCA TAG ACT GTA 3'	Koeleman et al.,2000
Int2-F	5' TTG CGA GTA TCC ATA ACC TG 3'	Koeleman et al.,2000
Int2-R	5' TTA CCT GCA CTG GAT TAA GC 3'	Koeleman et al.,2000

PCR production of this gene was performed by Int2-R, Int2-F, Int1-R, and Int1-F with size range varying from 160 to 288 bp. A PCR assay to detect the complete gene make up of class 1 integron was carried out in 20 μ l volume following the same concentration mixture mentioned earlier.

Statistical Analysis

Data were reported as mean \pm SD, frequency, and relative frequency. The Chi-

square test and Fisher exact test were used to investigate the association between categorical variables. P-values of less than 0.05 were considered statistically significant. SPSS version 19 and STATA version 11 were used for statistical analysis.

RESULTS

Of a total of 362 patients admitted to two ICUs (172 patients in General ICU and 190 patients in Toxicology ICU) and 55 and

120 samples were taken for microbiological culture from the General and Toxicology ICUs, respectively. Eventually, 40 Acinetobacter species were isolated (23 and 17 species from General and Toxicology ICUs, respectively). The mean±SD age of the patients was 50.47±21.32. Of the patients, 22 (55%) were and 18 (45%) were female. The characteristics of the patients categorized by the ICU type are shown in Table 2.

Table 2. The characteristics of patients with Acinetobacter by ICU type.

Characteristic	ICU Type					
	Toxicology n=17		General n=23		Total n=40	
	Frequency (%)		Frequency (%)		Frequency (%)	
Mental status	Conscious	2 (11.76)	9 (39.13)	11 (27.50)		
Ventilation		15 (88.24)	19 (86.36)	34 (87.18)		
Underlying disease		4 (23.53)	14 (60.87)	18 (45.00)		
	Decreased	3 (17.65)	6 (27.27)	9 (23.08)		
	Rale	8 (47.06)	4 (18.18)	12 (30.77)		
Lung Sound	Rhonchi	2 (11.76)	1 (4.55)	3 (7.69)		
	Normal	4 (23.53)	11 (50.00)	15 (38.46)		
Sputum	Yes	16 (94.12)	14 (60.87)	30 (75.00)		
sputum quality	Exudative	1 (5.88)	4 (17.39)	5 (12.50)		
	Non-exudative	16 (94.12)	19 (82.61)	35 (87.50)		
Urine culture	Positive	5 (29.41)	1 (4.35)	6 (15.00)		
	Negative	12 (70.59)	22 (95.65)	34 (85.00)		
Blood culture	Positive	1 (5.88)	5 (21.74)	6 (15.00)		
	Negative	16 (94.12)	18 (78.26)	34 (85.00)		
Tracheal	Positive	10 (58.82)	10 (43.48)	20 (50.00)		
	Negative	7 (41.18)	13 (56.52)	20 (50.00)		
Other culture	Positive	1 (5.88)	7 (30.43)	8 (20.00)		
	Negative	16 (94.12)	16 (69.57)	32 (80.00)		
	Non-integron	3 (17.65)	5 (21.74)	8 (20.00)		
	Integron1	2 (11.76)	1 (4.35)	3 (7.50)		
	Integron2	11 (64.71)	16 (69.57)	27 (67.50)		
	Integron1,2	1 (5.88)	1 (4.35)	2 (5.00)		
Outcome	Cure	9 (52.94)	11 (47.83)	20 (50.00)		
	Death	8 (47.06)	12 (52.17)	20 (50.00)		
	None	17 (100.00)	10 (43.48)	27 (67.50)		
	Hydrocephal	0 (.00)	2 (8.70)	2 (5.00)		
	Hypo Adenoma	0 (.00)	1 (4.35)	1 (2.50)		
	Artery Aneurism	0 (.00)	1 (4.35)	1 (2.50)		
	IVH	0 (.00)	0 (.00)	0 (.00)		
CVA	SAH	0 (.00)	1 (4.35)	1 (2.50)		
	Brain Aneurism	0 (.00)	1 (4.35)	1 (2.50)		
	Encephalite	0 (.00)	0 (.00)	0 (.00)		
	ICH	0 (.00)	7 (30.43)	7 (17.50)		
	None	15 (88.24)	16 (69.57)	31 (77.50)		
	Sepsis	2 (11.76)	2 (8.70)	4 (10.00)		
Infection	Bedsore	0 (.00)	1 (4.35)	1 (2.50)		
	Tetanus	0 (.00)	1 (4.35)	1 (2.50)		
	Colangitis	0 (.00)	3 (13.04)	3 (7.50)		
	None	17 (100.00)	20 (86.96)	37 (92.50)		
Acute surgery	Acute abdominal	0 (.00)	0 (.00)	0 (.00)		
	Mediastinitis	0 (.00)	1 (4.35)	1 (2.50)		
	Fistula	0 (.00)	1 (4.35)	1 (2.50)		
	Fracture	0 (.00)	1 (4.35)	1 (2.50)		
	Tracheostomy	5 (29.41)	6 (26.09)	11 (27.50)		

As it can be seen in Table 2, most of the isolated species have integron type 2. The antibiogram pattern is also shown in Table 2. The relationship between antibiogram resistance and integron was investigated. No significant relationship was seen between

resistances to the antibiotic used and integron type. Antibiogram resulted in isolated *Acinetobacter* and its association with integron are shown in Tables 3 and 4, respectively.

Table 3. Antibiogram results in isolated acinetobacter.

Antibiotic	Sensitive		Intermediate		Resistant	
	Frequency (%)		Frequency (%)		Frequency (%)	
Meropenem	0	(.00)	0	(.00)	40	(100.00)
Polymyxin	40	(100.00)	0	(.00)	0	(.00)
Gentamicin	13	(32.50)	1	(2.50)	26	(65.00)
Tetracycline	1	(2.50)	2	(5.00)	37	(92.50)
Imipenem	4	(10.00)	11	(27.50)	25	(62.50)
Ciprofloxacin	1	(2.50)	0	(.00)	39	(97.50)
Cotrimoxazole	0	(.00)	0	(.00)	40	(100.00)
Piperacillin	0	(.00)	0	(.00)	40	(100.00)
Piperacillin-tazobactam	0	(.00)	0	(.00)	40	(100.00)
Ceftazidime	0	(.00)	0	(.00)	40	(100.00)
Ceftriaxone	0	(.00)	0	(.00)	40	(100.00)
Cefepime	0	(.00)	0	(.00)	40	(100.00)
Cefotaxime	0	(.00)	0	(.00)	40	(100.00)

Table 4. Antibiogram results in isolated *Acinetobacter* by integron.

Antibiotic	Non-integron						Integron1					
	Sensitive		intermediate		resistant		sensitive		intermediate		resistant	
Number (percent)												
Polymyxin	8	(100)	0	(0)	0	(0)	3	(100)	0	(0)	0	(0)
Meropenem	0	(0)	0	(0)	8	(100)	0	(0)	0	(0)	3	(100)
Gentamicin	2	(25)	0	(0)	6	(75)	0	(0)	0	(0)	3	(100)
Tetracycline	1	(13)	0	(0)	7	(88)	0	(0)	0	(0)	3	(100)
Imipenem	0	(0)	2	(25)	6	(75)	0	(0)	0	(0)	3	(100)
Ciprofloxacin	0	(0)	0	(0)	8	(100)	0	(0)	0	(0)	3	(100)
Cotrimoxazole	0	(0)	0	(0)	8	(100)	0	(0)	0	(0)	3	(100)
Piperacillin	0	(0)	0	(0)	8	(100)	0	(0)	0	(0)	3	(100)
Piperacillin tazobactam	0	(0)	0	(0)	8	(100)	0	(0)	0	(0)	3	(100)
Ceftazidime	0	(0)	0	(0)	8	(100)	0	(0)	0	(0)	3	(100)
Ceftriaxone	0	(0)	0	(0)	8	(100)	0	(0)	0	(0)	3	(100)
Cefepime	0	(0)	0	(0)	8	(100)	0	(0)	0	(0)	3	(100)
Cefotaxime	0	(0)	0	(0)	8	(100)	0	(0)	0	(0)	3	(100)

Table 4. Antibigram results in isolated acinetobacater by integron (continued).

Antibiotic	Integrin 2			Intgrom1,2		
	sensitive	intermediate	resistant	sensitive	intermediate	resistant
	Number (percent)					
Polymyxin	27 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
Meropenem	0 (0)	0 (0)	27 (100)	0 (0)	0 (0)	2 (100)
Gentamicin	11 (41)	1 (4)	15 (56)	0 (0)	0 (0)	2 (100)
Tetracycline	0 (0)	2 (7)	25 (93)	0 (0)	0 (0)	2 (100)
Imipenem	4 (15)	9 (33)	14 (52)	0 (0)	0 (0)	2 (100)
Ciprofloxacin	1 (4)	0 (0)	26 (96)	0 (0)	0 (0)	2 (100)
Cotrimoxazole	0 (0)	0 (0)	27 (100)	0 (0)	0 (0)	2 (100)
Piperacillin	0 (0)	0 (0)	27 (100)	0 (0)	0 (0)	2 (100)
Piperacillin tazobactam	0 (0)	0 (0)	27 (100)	0 (0)	0 (0)	2 (100)
Ceftazidime	0 (0)	0 (0)	27 (100)	0 (0)	0 (0)	2 (100)
Ceftriaxone	0 (0)	0 (0)	27 (100)	0 (0)	0 (0)	2 (100)
Cefepime	0 (0)	0 (0)	27 (100)	0 (0)	0 (0)	2 (100)
Efotaxime	0 (0)	0 (0)	27 (100)	0 (0)	0 (0)	2 (100)

DISCUSSION

Nosocomial infections caused by multi-drug resistant *Acinetobacter* pose a serious problem in many countries. There are increasing reports of multidrug resistant *A. baumannii* (MDRAB) outbreaks in clinical settings worldwide [6,7,8] which require epidemiologic monitoring as a measure for controlling nosocomial infection [1].

The findings of this study about antibiotic resistance are compatible with many studies such as those by Japoni *et al.*, Mirnejad *et al.*, Peymani *et al.*, and others [4-8]. Our study found 100% antibiotic resistance patterns for meropenem, ceftazidime, ceftriaxone, cefepime, cotrimoxazole and piperacillin in accordance with CLSI 2011. This differs from resistance patterns found by Japoni *et al.* for meropenem (15.9%), cefepime (51.1%), and ceftazidime (52.3%) as well as 56% meropenem resistance in Peymani *et al.*'s study and 44% meropenem resistance in Mirnejad *et al.*'s study [5, 7]. The 97.5% resistance to ciprofloxacin in the present study can be compared to the 47.7% resistance in Japoni *et al.*'s study (2011) [4].

All data comparisons point to a growing resistance pattern over a short time.

In the present study, PCR was used for class 1 and 2 integron genes with primers Int2-R, Int2-F, Int1- R, and Int1-F 160 bp-288 bp. According to the base sequence of integrase genes, integrons included several classes. Class 1 integron was the most common among the clinical isolates of acinetobacter found in the Taherikalani's study (2011) while in the present study, class 2 integron was the most common among the clinical isolates of this microorganism (67.5%), closer to the findings of Mirnejad *et al.* (82%) [1, 5]. Integron 1 results in the present study (7.5%) significantly differed from the results reported by Japoni *et al.* (47.7%) and Peymani *et al.* (92.5%) [4, 7]. The non-integron results in the present study were 20% compared to 46.6% in Japoni *et al.*'s study[4].

Comparison of antibiotic resistance patterns and their association with class 1 and 2 integrons confirms that both classes of integrons exhibit similar resistance patterns to the tested antibiotics. However, class 1 integron is more likely to be involved in

emerging resistance to antibiotics [15]. Peymani's study also showed a significant association between integron carriage and reduced susceptibility to a variety of antibiotics, similar to studies done by Japoni and Falagas [4,16].

In the present study, no significant association was detected between resistance to antibiotics and the presence of integrons. This may indicate the role of other resistance determinants such as autolytic enzymes in the cell wall or plasmid or chromosomal super vision control which is compatible with recommendations in the studies done by Japoni *et al.* and Mirnejad *et al.* [4, 5] .

CONCLUSION

The use of an appropriate infection control strategy and a regular surveillance system seems warranted in hospitals in Iran.

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