

Prevalence of Class 1 Integron Among Multidrug-Resistant *Acinetobacter baumannii* in Tabriz, Northwest of Iran

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Abstract

Integrations are associated with a variety of gene cassettes, which confer resistance to multiple classes of antibacterial drugs. In this study we tested the frequency of class 1 and 2 integrons among multidrug-resistant *Acinetobacter baumannii* (MDRAB) clinical isolates. One hundred clinical isolates of *A. baumannii* were screened for carriage of class 1 and 2 integrons by PCR method. Results showed that seventy four (92.5%) of 80 MDRAB carried class 1 integron. Integron-positive isolates were statistically more resistant to aminoglycoside, quinolone and beta-lactam compounds except for cefepime. This is the first report of class 1 integrons in MDRAB isolates in northwest Iran.

Key words: *Acinetobacter baumannii*, class 1 integron, multidrug resistance

Acinetobacter baumannii (*A. baumannii*) is one of the most important nosocomial pathogens that causes various types of clinical infections (Dinc *et al.*, 2010). This organism is ranked second after *Pseudomonas aeruginosa* among gram-negative nosocomial pathogens (Berlau *et al.*, 1999). *A. baumannii* is becoming a serious clinical concern due to its noticeable ability to develop resistance to several classes of antimicrobial drugs. There are increasing reports of multidrug-resistant *A. baumannii* (MDRAB) outbreaks in clinical settings worldwide (Peleg *et al.*, 2008). The MDRAB term was defined as those isolates that are intermediate or resistant to at least three different classes of antimicrobial agents mainly beta-lactams, aminoglycosides, fluoroquinolones and carbapenems (Huang *et al.*, 2008). Carbapenems are currently the drugs of choice for treatment of severe infections caused by this organism. However, resistance to carbapenems in this species is now reported increasingly throughout the world (Marais *et al.*, 2004; Merkier *et al.*, 2008; Dijkshoorn *et al.*, 2007). Studies have shown that MDRAB may be developing through the acquisition or horizontal transfer of the antibiotic resistance genes. Integrons are mobile genetic elements capable to cap-

ture and speared of antimicrobial resistance genes. To date, several classes of integrons have been described among them class 1 and 2 integrons are the most frequently identified integrones in clinical isolates of *A. baumannii* that confer resistance to multiple classes of antimicrobial drugs (Oh *et al.*, 2002). The aims of this study were to determine the frequency of class 1 and 2 integrons among MDRAB clinical isolates and to investigate the association between the presence of integrons and antibiotic resistance patterns in MDRAB isolates.

This study was carried out at a 1000 beds tertiary care hospital in Tabriz, Northwest Iran. Written informed consent was obtained from all subjects enrolled in this study and the protocol was approved by the Regional Ethical Committee. A total of 100 non-duplicated clinical isolates were collected during a period of 16 months between March 2008 and June 2009 from patients admitted to hospital. 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 (Feizabadi *et al.*, 2008). Species identification was confirmed by detection of *bla*_{OXA-51-like} gene as described previously (Turton *et al.*, 2006).

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Antimicrobial susceptibility test was performed using the standard Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guideline. The antimicrobial agents tested were ampicillin-sulbactam (10/10 µg), piperacillin-tazobactam (100/10 µg), amoxicillin/clavulanic acid (20/10 µg), ticarcillin/clavulanic acid (75/10 µg), ceftriaxone (30 µg), cefepime (30 µg), ceftazidime (30 µg), piperacillin (100 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), amikacin (30 µg) and trimethoprim/sulfamethoxazole (25 µg). All antibiotics disks were purchased from Mast, UK. The Minimum Inhibitory Concentration (MICs) of imipenem and meropenem were determined by the E-test method (AB Biodisk, Solna, Sweden) according to the CLSI guidelines. *Pseudomonas aeruginosa* ATCC 27853 was used as the control strain in antimicrobial susceptibility testing. Extraction of genomic DNA from *A. baumannii* isolates was performed by boiling method. PCR assay was performed for detection of class 1 and 2 integron genes using the following consensus primer pairs: *Int1*-F: 5'-GGTGTGGCGGGCTTCGTG-3', *Int1*-R: 5'-GCATCCTCGGTTTTCTGG-3', *Int2*-F: 5'-CAC GGATATGCGACAAAAAGGT-3' and *Int2*-R: 5'-GTAGCAAACGAGTGACGAAATG-3' (Shibata *et al.*, 2003). The primers were purchased from MWG Company, Germany. *P. aeruginosa* ATCC 27853 was used as the negative control and *P. aeruginosa* harboring integrons class 1 and 2 genes (kindly provided from Dr Shahcheraghi, Iran) were used as positive controls. Descriptive statistics including frequencies, crosstabulation of microbiological, clinical and demographic characteristics were performed using the software program SPSS, version 16 (SPSS). The chi-square test was used to determine the association between integron carriage and antimicrobial susceptibility patterns. A *P* value of less than 0.05 was considered statistically significant.

A total of 100 clinical isolates were collected from patients admitted to different wards of Imam Reza hospital of Tabriz, Iran. The isolates were obtained from different clinical specimens including trachea (37%), urine (21%), sputum (9%), blood (7%), catheter (6%), bronchial washings (6%), wound (5%), abscess drainage (3%), cerebrospinal fluid (2%), ascitic fluid (2%) and pleural effusion (2%). The isolates were obtained from patients in intensive care units (38%), internal (24%), neurosurgery (17%), infectious (9%), neurology (8%) and general surgery (3%) wards. Seventy two patients (72%) were males and 28 (28%) were females. Their ages ranged from 14 to 86 years with a mean of 51 years. Analysis for presence of *bla*_{OXA-51-like} gene revealed that all isolates were positive for *bla*_{OXA-51-like} gene that confirmed their identity as *A. baumannii*. Among one hundred isolates that studied, 80 isolates exhibited the

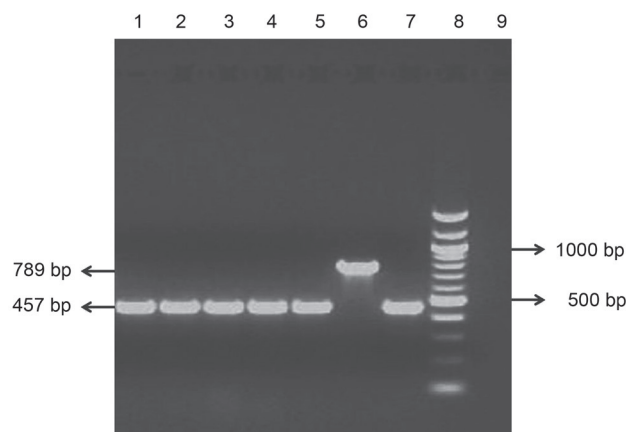


Fig. 1. PCR amplification of class 1 and 2 integron genes among clinical isolates of *A. baumannii* isolated from Imam Reza hospital of Tabriz, Iran

Lane 1-5: Positive clinical *A. baumannii* isolates for class 1 integron; Lane 6: Positive control for class 2 integron; Lane 7: Positive control for class 1 integron; Lane 8: 100 bp molecular size marker; Lane 9: Negative control

MDR pattern. Seventy four (92.5%) of MDR isolates were found to have the class 1 integron, whereas only 6 isolates (7.5%) containing class 1 integron were non-MDR. Class 2 integron gene was not found in this study (Fig. 1). Determination of MICs against imipenem and meropenem by E-test method showed that mean MICs among the MDR isolates were 26.11 mg/l and 29.88 mg/l while this value for non-MDR isolates were 1.85 mg/l and 2.12 mg/l, respectively. The antimicrobial susceptibility of integron-positive and integron-negative isolates and the association between the presence of class 1 integron and antibiotic resistance patterns are shown in Table I. Among 80 class 1 integron-positive isolates, 56 (70%) and 54 (67.5%) isolates were meropenem and imipenem resistant, respectively. Analysis of data revealed a significant association between MDR pattern and presence of class 1 integron ($p < 0.001$). The results also showed that integron-positive isolates were statistically more resistant to aminoglycoside, quinolone and beta-lactam compounds, except for cefepime. There was no association between the presence of class 1 integron and resistance to ampicillin and cefpodoxime, to which all isolates were fully resistant.

The present study showed that class 1 integron is widely distributed among MDRAB isolates in our hospital. This rate (92.5%) is considerably higher than the rates found in other geographical regions including European countries (43%) (Martinez-Freije *et al.*, 1998), United Kingdom (60%) (Turton *et al.*, 2005) and China (51.9%) (Gu *et al.*, 2007). Our study also revealed that class 1 integron was significantly associated with resistance to given antibiotics, including aminoglycosides, quinolones, and beta-lactam compounds. This is not surprising, since class 1 integron carry many antibiotic resistance gene cassettes which

Table I

Antibiotic susceptibility profile of integron-positive and integron-negative *A. baumannii* isolates from Imam Reza hospital, Tabriz, Iran

Anti-biotics	Antibiotic susceptibility			Integron-positive isolates			Integron-negative isolates			P value
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	
ATM	94	2	4	74	0	2	20	2	2	0.017
CRO	91	3	6	74	1	1	17	2	5	<0.001
CTX	93	3	4	76	0	0	17	3	4	<0.001
CAZ	92	3	5	73	1	2	19	2	3	0.029
PIP	91	1	8	75	0	1	16	1	7	<0.001
TZP	88	3	9	74	1	1	14	2	8	<0.001
AMC	88	6	6	73	1	2	15	5	4	<0.001
SXT	86	3	11	72	1	3	14	2	8	<0.001
FEB	85	3	12	68	1	7	17	2	5	0.55
GEN	85	5	10	69	4	3	16	1	7	0.002
CIP	85	4	11	72	1	3	13	3	8	<0.001
LVX	83	2	3	71	2	3	12	0	12	<0.001
TIM	82	8	10	67	5	4	15	3	6	0.009
AMK	80	2	18	67	2	7	13	0	11	<0.001
SAM	60	21	19	51	18	7	9	3	12	0.001
MEM	56	6	38	50	6	20	6	0	18	<0.001
IPM	53	6	41	49	5	22	4	1	19	<0.001

Abbreviations: R – Resistance; I – Intermediate; S – Sensitive; ATM – Aztreonam; CRO – Ceftriaxone; CTX – Cefotaxime; CAZ – Ceftazidime; PIP – Piperacillin; TZP – Piperacillin-tazobactam; AMC – Amoxicillin-clavulanic acid; SXT – Trimethoprim-sulfamethoxazole; FEP – cefepime; GEN – Gentamicin; CIP – ciprofloxacin; LVX – Levofloxacin; TIM – Ticarcillin-clavulanic acid; AMK – Amikacin; SAM – Ampicillin-sulbactam; MEM – Meropenem; IPM – Imipenem

encode resistance to a wide range of antibiotics in *Acinetobacter* spp. (Perez *et al.*, 2007). However, 7.5% of integron-negative isolates were also resistant to these drugs. The antibiotic resistance genes of these isolates could be acquired by chromosomal-encoded enzymes or other mobile elements.

Most of integron positive isolates were resistant to aminoglycoside compounds. This could be explained by the fact that many gene cassettes conferring resistance to aminoglycosides (*aad* and *aac* genes) are carried on class 1 integron structures (Nemec *et al.*, 2004). This study also showed the high resistance rate to several classes of beta-lactam compounds in integron-positive strains indicating carriage of several beta-lactamase encoding genes by class 1 integron. More importantly, TEM- or SHV- and OXA-types carbapenemase and metallo-beta-lactamase enzymes might have contributed in resistance to carbapenems, extended spectrum cephalosporins, most penicillins and penicillin-beta-lactamase inhibitor compounds (Liu *et al.*, 2006). However, we found no association between integron presence and nonsusceptibility to cefepime in MDRAB isolates suggesting that other antibiotic resistance mechanisms may be involved in resistance to this drug in our strains.

This study revealed a significant association between the presence of the class 1 integron and quinolone resis-

tance. This is perhaps surprising since resistance to quinolone compounds is often results from chromosomal point mutations. However, it has been reported that integrons or associated plasmids may harbor genes encoding proteins that affect the permeability of cells or the efflux systems conferring resistance to some quinolone and betalactam compounds (Vila *et al.*, 2007). In conclusion, this study showed high prevalence of class 1 integron among *A. baumannii* isolated from our hospital settings. Considering the significant association between integron carriage and reduced susceptibility to variety of antibiotics, use of appropriate infection control strategy and a regular surveillance system is necessary in our hospital. Nevertheless, further studies need to be carried out to characterize different resistance gene cassettes within integron class 1 in these isolates.

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