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Characterized of class I integron in *Acinetobacter baumannii* isolated from upper respiratory tract

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Abstract: [Objective] To characterize the class I integron in *Acinetobacter baumannii* and to analyze the correlation between integron and drug resistance. **[Methods]** In total 187 strains were collected between 2008 and 2009. All strains were tested by Kirb-Bauer disk diffusion test for drug resistance. PCR and DNA sequencing were used to detected class I integrase gene and to clarify the context of gene cassette. **[Results]** Class I integrase gene was detected in 100 (53.4%) of the isolates analyzed. Seven different gene cassettes were identified, including a new integron (GenBank: HQ322622) carrying an unknown protein probably associated with recombination. The vast majority of the cassettes encoded amonoglycoside resistance gene, including aacA4, aadA1, aacC1, aac6 II, aadA2. Susceptibility data show that strains carrying class I integron were significantly more resistant to all of the antibiotics tested than isolates lacking class I integron. The correlation between the presence of integron and the multidrug-resistance of *A. baumannii* was statistically significant. **[Conclusion]** Drug resistance genes integrated by Class I integron were widespread in *A. baumannii*. Class I integron plays an important role in resistance of *A. baumannii*.

Keywords: multi-drug resistance, *Acinetobacter baumannii*, class I integron
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Acinetobacter baumannii is a glucose-nonfermentative gram-negative coccobacillus that is an important opportunistic pathogen. Because of the multiple antibiotic resistance exhibited by *A. baumannii*, nosocomial infections caused by this organism are difficult to treat [1-2]. In recent years, several outbreaks of nosocomial infections caused by *A. baumannii* have been documented [3,4]. Studies of antibiotic resistance mechanisms in *A. baumannii* have demonstrated the presence of specific genes located on integron [5-6]. Integron form an important source for the spread of antibiotic resistance, especially in gram-negative bacteria [7-8]. An integron includes a gene encoding an integrase flanked by an *attI* recombination site. Gene cassettes are not necessarily part of the

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integron, but when integrated, they become part of the integron, often comprising antibiotic resistance genes[7]. The presence of class Ⅰ and class Ⅱ integron have already been described in *A. baumannii* strains of both clinical and environmental origin. Class Ⅰ integron was the most prevalent among *A. baumannii*.

The purposes of the present study were to investigate the molecular characteristics of class Ⅰ integron and gene cassette arrays in *A. baumannii* strains during the last two years.

1 Materials and Methods

1.1 Isolates

Between January 2008 and December 2009, a total of 187 non-repetitive clinical isolates of *A. baumannii* were collected from the Guangzhou Institute of Respiratory Disease. All of the strains isolated from sputum. *A. baumannii* isolates resistant to three or more different classes of antibiotics, including at least one extended- spectrum β-lactam antibiotic, were defined as multi-drug resistant *A. baumannii* (MDR-AB)[8]. All clinical isolates were identified using the VITEK2-compact 30 system (bioMerieux).

1.2 Antimicrobial susceptibility testing

The isolates were screened for antimicrobial susceptibility using the Kirb-Bauer disk diffusion test methodology. The following antibiotics were tested: ampicillin (AM, 10 μg), piperacillin (PIP, 100 μg), ticarcillin/clavulanic acid (TIM, 75/10 μg), piperacillin/tazobactam (TZP, 100/10 μg), ampicillin/sulbactam (SAM, 10/10 μg), cefoperazone/sulbactam (CFPS, 75/75 μg), ceftriaxome (CRO, 30 μg), cefotaxime (CTX, 30 μg), cefepime (FEP, 30μg), ceftazidime (CAZ, 30 μg), imipenem (IPM, 10μg), meropenem (MEM, 10μg), aztreonam (ATM, 30 μg), amikacin (AN, 30 μg), gentamicin (GM, 10 μg), minocycline (MI, 30 μg), levofloxacin (LVX, 5 μg), ciprofloxacin (CIP, 5μg), moxifloxacin (MXF, 5 μg), trimethoprim-sulfamethoxazole (SXT, 1.25/ 23.75 μg). Two control strains of *E. coli* (ATCC25922) and *P. aeruginosa* (ATCC27853) were included in the test. The sensitivity was determined by the criteria of the recommendation of the clinical laboratory standards institute 2007 (CLSI).

1.3 Integron detection and typing

Detection of class Ⅰ integrons was carried out by PCR amplification of an internal fragment within the class Ⅰ integrase gene using the primers int1-F (5′-GCTTACGAACCGAACAGGC), int1-R (5′-CCGAGGATGCGAACCACT). To characterize inserted gene cassettes, the variable regions of class Ⅰ integrons were amplified with primers 5′-CS/3′-CS according to the method previously[6]. PCR products with the same size were digested with 15U of *Hea* Ⅲ (New England Biolabs, USA) and 25U of *Hinf* Ⅰ at 37℃ 2hr. After restriction digestion, the fragments were resolved by electrophoresis at 90V for 45min on 2% agarose gels with 0.5 × TBE buffer. Then gels were stained by ethidium bromide and were visualized under UV light. The variable regions showing different sizes or different digestion profiles were subsequently purified from agarose gels and subcloned using the T simple vector (TaKaRa, Japan). The ligation mixes were transformed into *E. coli* DH5α, and then selecting with 50μg/ml ampicillin MacConkey agar plates. Recombinant plasmid DNA was purified using Qiaquick purification columns (QIAGEN, USA) according to the standard methods and subjected to sequencing on ABI3730xl DNA analyzer (Applied Biosystems, USA). Additional primers were designed on the basis of sequences obtained to complete the entire sequence of the gene cassettes. The nucleotide sequences were analyzed and compared with those in GenBank using the BLAST algorithm (http://www.ncbi.nlm.nih.gov)

1.4 Statistical anaylsis

The chi-square test was used to determine the significance of differences. A difference was considered statistically significant if the p value was less than 0.05. Bivariate correlation between MDR-AB and integron were calculated via spearman′s rank correlation coefficients.

2 Results

2.1 Antimicrobial resistance

Along the 187 isolates analyzed by PCR, class1 integrase gene was detected in 100 isolates (53.48%). Isolates carrying class1 integrase gene were significantly more resistant to all of the antibiotics tested than isolates lacking class1 integrase gene ($P < 0.008$) (Table 1). Along 187 isolates, 40 strains were MDR-AB. The correlation between the presence of integron and the MDR-AB was statistically significant ($P < 0.05$).

Table 1 Susceptibility testing results of integron-positve and integron-negative *Acinetobacter baumannii* isolates

Antibiotic	Antibiotic susceptibility (n = 187)			Integron-positve isloates (n = 100)			Integron-negative isloates (n = 87)			P-value [a]
	%R (no.)	%I (no.)	%S (no.)	%R (no.)	%I (no.)	%S (no.)	%R (no.)	%I (no.)	%S (no.)	
AM	68.45 (128)	0.53 (1)	31.02 (58)	97 (97)	0 (0)	3 (3)	35.63 (31)	1.15 (1)	63.22 (55)	<0.001
PIP	67.38 (126)	6.42 (12)	26.20 (49)	96 (96)	0 (0)	4 (4)	34.48 (30)	13.79 (12)	51.72 (45)	<0.001
TIM	61.50 (115)	5.88 (11)	32.62 (61)	93 (93)	3 (3)	4 (4)	25.29 (22)	9.20 (8)	65.52 (57)	<0.001
TZP	60.43 (113)	6.95 (13)	32.62 (61)	88 (88)	6 (6)	6 (6)	28.74 (25)	8.05 (7)	63.22 (55)	<0.001
SAM	19.79 (37)	3.21 (6)	77.01 (144)	12 (12)	5 (5)	83 (83)	28.74 (25)	1.15 (1)	70.11 (61)	0.008
CFPS	14.97 (28)	20.32 (38)	64.71 (121)	19 (19)	31 (31)	50 (50)	10.34 (9)	8.05 (7)	81.61 (71)	<0.001
CRO	62.57 (117)	32.62 (61)	4.81 (9)	95 (95)	4 (4)	1 (1)	25.29 (22)	65.52 (57)	9.20 (8)	<0.001
CTX	64.17 (120)	20.86 (39)	14.97 (28)	95 (95)	3 (3)	2 (2)	28.74 (25)	41.38 (36)	29.89 (26)	<0.001
FEP	45.45 (85)	17.11 (32)	37.43 (70)	64 (64)	24 (24)	12 (12)	24.14 (21)	9.20 (8)	66.67 (58)	<0.001
CAZ	60.43 (113)	0.53 (1)	39.04 (73)	93 (93)	1 (1)	6 (6)	22.99 (20)	0.00 (0)	77.01 (67)	<0.001
IPM	36.36 (68)	1.60 (3)	62.03 (116)	51 (51)	3 (3)	46 (46)	19.54 (17)	0.00 (0)	80.46 (70)	<0.001
MEM	31.02 (58)	4.28 (8)	64.71 (121)	42 (42)	8 (8)	50 (50)	18.39 (16)	0.00 (0)	81.61 (71)	<0.001
ATM	79.68 (149)	15.51 (29)	4.81 (9)	93 (93)	6 (6)	1 (1)	64.37 (56)	26.44 (23)	9.20 (8)	<0.001
AN	55.61 (104)	0.53 (1)	43.85 (82)	93 (93)	0 (0)	7 (7)	12.64 (11)	1.15 (1)	86.21 (75)	<0.001
GM	62.57 (117)	1.60 (3)	35.83 (67)	98 (98)	0 (0)	2 (2)	21.84 (19)	3.45 (3)	74.71 (65)	<0.001
MI	38.50 (72)	21.93 (41)	39.57 (74)	65 (65)	33 (33)	2 (2)	8.05 (7)	9.20 (8)	82.76 (72)	<0.001
LVX	63.10 (118)	5.35 (10)	31.55 (59)	88 (88)	9 (9)	3 (3)	34.48 (30)	1.15 (1)	64.37 (56)	<0.001
CIP	67.38 (126)	2.67 (5)	29.95 (56)	97 (97)	1 (1)	2 (2)	33.33 (29)	4.60 (4)	62.07 (54)	<0.001
MXF	64.71 (121)	3.21 (6)	32.09 (60)	95 (95)	2 (2)	3 (3)	29.89 (26)	4.60 (4)	65.52 (57)	<0. <0.001
SXT	64.71 (121)	1.07 (2)	34.22 (64)	97 (97)	0 (0)	3 (3)	27.59 (24)	2.30 (2)	70.11 (61)	<0 <0.001

AM, ampicillin; PIP, piperacillin; TIM, ticarcillin-clavulanic acid at a 7.5:1; TZP, piperacillin with a fixed concentration of tazobactam at 4mg/L; SAM, ampicillin-sulbactam at a 2:1 ration; CFPS, cefoperazone; CRO, ceftriaxome; CTX, cefotaxime; FEP, cefepime; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; ATM, aztreonam; AN, amikacin; GM, gentamicin; MI, minocycline; LVX, levofloxacin; CIP, ciprofloxacin; MXF, moxifloxacin; SXT, trimethoprim-sulfamethoxazole.

[a] the chi-square test was used to calculate the P value in terms of resistant, intermediate, and susceptible numbers of integron-positive and - negative isolates.

A p-value of <0.05 is considered to indicate a significant difference in resistance between integron-positve and integron-negative isolates.

2.2 Characterization class I integrons

To further investigate the mechanisms of antibiotic resistance in *A. baumannii*, isolates were analyzed for class Ⅰ integron variable region. The variable regions were amplified with the primers 5′ CS and 3′ CS, which annealed with DNA regions flanking the recombination site. Amplification of these int1-positive isolates gave PCR products of various sizes, approximately3.0, 2.4, 2.2, 1.5 and 1.0 kb. To further differentiate between the gene cassette amplification products, they were digested with *Hea* Ⅲ and *Hinf* Ⅰ. After endonuclease analysis and sequencing analysis of the amplification of the integron gene cassettes, all these gene cassettes were divided into seven different gene cassettes (Table 2). The most prevalent type of cassette was class Ⅰ, accounting for 75% of all cassettes. The type 7 was firstly discovered (Fig. 1).

Table 2 Details of integron cassette arrays sequenced and accession numbers of matching integrons found in *Acinetobacter baumannii* isolates

Integron type	Sequenced size(5′ CS to 3′ CS) bp	No of isolates	Gene cassette(s) and order	GenBank
1	2380	75	aacA4-catB8-aadA1	AY922989.1
2	3080	16	aacC1-*orf*X-*orf*X-*orf*X′-aadA1	AY577724.1
3	1395	5	arr3-aacA4	AY038837.3
4	1636	1	aadA1-aac6 Ⅱ	DQ402099.1
5	1009	1	aadA2	GU001948.1
6	951	1	fhaC	FM955483.1
7	2168	1	Unkown	HQ322622

3 Discussion

Among all integron positive isolates, seven integron cassette types were identified (Table 2). Type1, 2, 3 cassette have been previously documented in *A. baumannii*[6, 10], type 4 cassette has appeared in *Aeromonas hydrophila* (GenBank: DQ402099.1), type 5 cassette have been identified in *Escherichia coli* (GenBank: GU001948.1), *Salmonella typhimurium* (GenBank: GU987052.1) and *Enterobacter aerogenes* (GenBank: FJ004895.1), type 6 have been described in bacterial isolated from Indian river (GenBank: FM955483.1). To our knowledge, type 4, 5 and 6 was the first time described in *A. baumannii*. Aminoglycoside resistance determinants including aacA4, aadA1, aaţC1, aac6 Ⅱ, aadA2, were predominantly found in our study. The high prevalence of aminoglycoside resistance genes in the *A. baumannii* integron has been also observed in other studies[6, 11].

Interestingly, type 7 cassette (GenBank: HQ322622) was a novel gene cassette, which probably encoded a helicases involved in recombination. Type 7 cassette was 2168bp, which have no similarity sequence in NCBI database. We use Open Reading Frame Finder software (http://www.ncbi.nlm.nih.gov/projects/gorf/) predict the putative coding region, found the variable region of type 7 cassette encoded a protein has 53% similarity to type Ⅲ restriction protein res subunit (GenBank: abk49189.1) and type I restriction-modification system R subunit (GenBank: AAN57238.1). Predicted protein of type 7 cassette have two conserved domanins, one was DEAD-like helicases superfamily, another was P-loop containing nucleoside triphosphate hydrolases, both of them are involved in ATP-dependent RNA or DNA unwinding (Fig. 1). So we speculated that class Ⅰ integron may be involved in getting other function genes, like recombination.

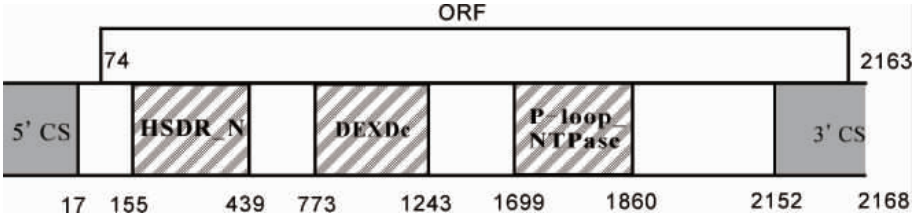

Fig. 1 Schematic structures of the type 7 integron. The number indicated the sequence of nucleotide. Gray box represent clone primer; striped box represent domains. HSDR_N is the abbreviation of I restriction enzyme R protein N terminus; DEXDc is the abbreviation of DEAD-like helicases superfamily; P-loop_NTPase is the abbreviation of P-loop containing Nucleoside Triphosphate Hydrolases.

Strains carrying Class Ⅰ integron were significantly more resistant to all of the antibiotics tested then isolates lacking Class Ⅰ integron, which indicates that integron may play an important role in reisistance of *A. baumannii*. The correlation between the presence of integron and the MDR-AB was statistically significant ($P < 0.05$), indicated encoding class Ⅰ integron was strongly correlated with multi-drug resistance phenotype.

REFERENCES

[1] Bergogne-Berezin E, Towner KJ. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clinical Microbiology Reviews*, 1996, 9(2):148-165.

[2] Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. *Natuer Review Microbiology*, 2007, 5(12): 939-951.

[3] Enoch DA, Summers C, Brown NM, Moore L, Gillham MI, Burnstein RM, Thaxter R, Enoch LM, Matta B, Sule O. Investigation and management of an outbreak of multidrug-carbapenem-resistant Acinetobacter baumannii in Cambridge, UK. *Journal of Hospital Infection*, 2008, 70(2):109-118.

[4] Chang HL, Tang CH, Hsu YM, Wan L, Chang YF, Lin CT, Tseng YR, Lin YJ, Sheu JJ, Lin CW. Nosocomial outbreak of infection with multidrug-resistant Acinetobacter baumannii in a medical center in Taiwan. *Infection Control and Hospital Epidemiology*, 2009, 30 (1):34-38.

[5] Gu B, Tong M, Zhao W, Liu G, Ning M, Pan S, Zhao W. Prevalence and characterization of class I integrons among Pseudomonas aeruginosa and Acinetobacter baumannii isolates from patients in Nanjing, China. *Journal of Clinical Microbiology*, 2007, 45 (1): 241-243.

[6] Turton JF, Kaufmann ME, Glover J, Coelho JM, Warner M, Pike R, Pitt TL. Detection and typing of integrons in epidemic strains of Acinetobacter baumannii found in the United Kingdom. *Journal of Clinical Microbiology*, 2005, 43(7):3074-3082.

[7] Mazel D. Integrons: agents of bacterial evolution. *Natuer Review Microbiology*, 2006, 4(8):608-620.

[8] Martinez-Freijo P, Fluit AC, Schmitz FJ, Grek VS, Verhoef J, Jones ME. Class I integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. *Journal of Antimicrobial Chemotherapy*, 1998, 42(6):689-696.

[9] Falagas ME, Koletsi PK, Bliziotis IA. The diversity of definitions of multidrug-resistant (MDR) and pandrug-resistant (PDR) Acinetobacter baumannii and Pseudomonas aeruginosa. *Journal of Medical Microbiology*, 2006, 55(12):1619-1629.

[10] Huang LY, Chen TL, Lu PL, Tsai CA, Cho WL, Chang FY, Fung CP, Siu LK. Dissemination of multidrug-resistant, class 1 integron-carrying Acinetobacter baumannii isolates in Taiwan. *Clinical Microbiology and Infection*, 2008, 14(11):1010-1019.

[11] Chiu CH, Lee HY, Tseng LY, Chen CL, Chia JH, Su LH, Liu SY. Mechanisms of resistance to ciprofloxacin, ampicillin/sulbactam and imipenem in Acinetobacter baumannii clinical isolates in Taiwan. *International Journal of Antimicrobial Agents*, 2010, 35(4):382-386.

上呼吸道分离鲍曼不动杆菌 1 型整合子的分离与鉴定

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摘要:【目的】研究 I 型整合子的结构特征,探讨其与细菌多重耐药之间的相关性。**【方法】**收集 2008 年至 2009 年广州呼吸疾病研究所上呼吸道分离的 187 株鲍曼不动杆菌,应用 K-B 纸片扩散法检测耐药性,采用聚合酶链式反应进行 I 型整合子整合酶基因的检测;扩增整合子的可变区,应用 DNA 测序技术分析 I 型整合子基因结构。**【结果】**I 型整合子的阳性率达 53.4%。共七种 1 型整合子基因盒被鉴定,其中首次发现报道一种新的整合子(GenBank:HQ322622)。可变区主要编码氨基糖苷类药物的耐药基因。20 种抗菌素耐药的结果均表明携带 I 型整合子的鲍曼不动杆菌耐药率较不携带 I 型整合子的鲍曼不动杆菌的耐药率明显增高。整合子与鲍曼不动杆菌的多重耐药表型具有密切相关性。**【结论】**I 类整合子相关耐药基因在本院临床分离鲍曼不动杆菌中分布较广泛。整合子在鲍曼不动杆菌耐药性的形成和播散中具有重要作用。

关键词: 多重耐药,鲍曼不动杆菌,I 型整合子

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《微生物学报》综述文章投稿最新要求

2011 年 12 月,第 3 次修订

为了避免篇幅庞大、罗列文献、内容空泛、缺乏观点,力求内容更加新颖、并更具可读性,自 2003 年本刊对综述类投稿提出了具体的要求,先后又作了两次修订。

1. 篇幅:主要刊登微型综述(mini review),来稿字数最好控制在 5000 字以内(不包括参考文献)。
2. 新意:选题要有新意,对读者及同行确有一定的启发作用和参考价值。
3. 述和评:结合文献扼要评述国内外学者在本领域的研究进展,不要泛泛罗列文献,只述不评。
4. 结合工作:结合自己的研究工作,就该研究领域存在的问题和解决的途径提出自己的观点。
5. 参考文献:控制在 40 篇以内,近 3 年发表的文献不少于 10 篇。
6. 作者:(1)数量不多于 3 人;(2)提供一份背景材料,内容包括:第一作者科研简介、责任作者(即通讯作者)科研简介、本课题组对相关工作情况的介绍(附已发文章)。

欢迎投送“能够反映国际研究热点、对学科发展有指导意义”的述评类文章。