CLASS 1 INTEGRONS IN *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII* ISOLATED FROM CLINICAL ISOLATES

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Abstract. Resistance to various antimicrobial agents is an increasing problem in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections. In this study, the roles of integrons were examined in 101 *P. aeruginosa* isolates and 176 *A. baumannii* isolates from patients. The frequencies and characteristics of class 1, 2 and 3 integrons were investigated and the horizontal transfer of integrons was assessed. Among these isolates, class 1 integrons with a resistance gene cassette were detected in 69.3% of *P. aeruginosa* and 31.8% of *A. baumannii* isolates, but class 2 and 3 integrons were not found. Five novel gene cassette arrays were identified in *P. aeruginosa*: *aacA7-cmlA*, *aadB-bla*$_{OXA-10}$*-aadA1*, *aadB-arr-2-cmlA-bla*$_{OXA-10}$*-aadA1*, *aadB-cmlA-aadA1* and *aadB-cmlA-bla*$_{OXA-10}$*-aadA15*. The integrons found in *A. baumannii* isolates in this study were previously reported. Horizontal transfer of some class 1 integrons was detected in both *P. aeruginosa* (2/70) and *A. baumannii* (5/57). These data confirm the high prevalence of class 1 integrons with a variety of gene cassette combinations among multidrug-resistant *P. aeruginosa* and *A. baumannii* clinical isolates.

Keywords: *Acinetobacter baumannii*, class 1 integrons, multidrug resistance, *Pseudomonas aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa and *Acinetobacter baumannii* are recognized as common nosocomial pathogens. They are clinically significant due to their multidrug-resistant (MDR) phenotypes leading to therapeutic failures. Several resistance mechanisms have been identified in these two pathogens, including acquisition of resistance-encoding genes through mobile genetic elements (Seward, 1999; Xu *et al*, 2009). These elements include integrons able to integrate and mobilize gene cassettes, most of which contain resistance-encoding genes (Fluit and Schmitz, 1999). To date, nine classes of integrons have been recognized, among which class 1 integrons are the most prevalent among *P. aeruginosa* and *A. baumannii* (Gu *et al*, 2007). Of particular concern are class 1 integrons frequently located in plasmids and transposons. These have the ability to undergo horizontal transfer and contribute to rapid dissemination of antibiotic

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resistance genes among bacterial isolates not limited to *P. aeruginosa* and *A. baumannii* (Fluit and Schmitz, 1999).

Resistance to various antibiotics is common among *P. aeruginosa* and *A. baumannii* isolates in many parts of the world, including Thailand. However, there is a relative paucity of data on integron-associated gene cassettes among MDR *P. aeruginosa* and *A. baumannii* strains, particularly in developing countries. This study was conducted to characterize antibiotic susceptibilities and class 1 integrons among *P. aeruginosa* and *A. baumannii* isolates. The presence of class 2 and 3 integrons was also investigated.

MATERIALS AND METHODS

Bacterial isolates and antimicrobial susceptibility testing

One hundred one *P. aeruginosa* isolates and 176 *A. baumannii* isolates were randomly selected from the stock of Siriraj Hospital, Bangkok Thailand. The specimens were obtained from a variety of clinical isolates collected during 2001-2008. All bacterial strains were identified using the VITEK GNI card (bioMérieux Vitek, Hazelwood, MO) and the API 20NE system (bioMérieux). One colony was collected from each positive clinical sample. The genetic relatedness of these isolates was not tested. All isolates were tested for minimum inhibitory concentrations (MICs) of 15 antimicrobial agents: amikacin (Amk), aztreonam (Atm), carbenicillin(Car), ceftaxidime (Cef), chloramphenicol (Chp), ciprofloxacin(Cip), erythromycin (Ery), gentamicin (Gen), kanamycin (Kan), neomycin (Neo), piperacillin (Pip), streptomycin (Str), spectinomycin (Spc), tetracycline (Tet) and trimethroprim (Tri), using a two-fold agar dilution method, according to CLSI guidelines (CLSI) (NCCLS, 1998).

P. aeruginosa ATCC27853 strain was used as a control. Multidrug resistance was defined as resistance to at least 6 different antimicrobial agents (Gu *et al*, 2007).

PCR, DNA purification and DNA sequencing

Template DNA used for PCR was prepared as previously described (Levesque *et al*, 1995). All *P. aeruginosa* and *A. baumannii* isolates were screened for the presence of integrase genes *intI1* and *intI2* and *intI3* using the following primer pairs: for *intI1*, int1LF (5′-CAG GAG ATC GGA AGA CCT-3′) and int1LR (5′-TTG CAA ACC CTC ACT GAT-3′); for *intI2*, (5′-GGC AGA CAG TTG CAA GAC AA -3′) and (5′-AAG CGA TTT TCT GCG TGT TT-3′) and for *intI3*, (5′-CCG GTT CAG TCT TTC CTC AA-3′) and (5′-GAG GCG TGT ACT TGC CTC AT-3′) (Chuanchuen *et al*, 2007; Ekkapobyotin *et al*, 2008). Inserted-gene cassettes were analyzed using PCR with a conserved segment primer set: 5′CS-GGCATCCAAGCAGCAAG and 3′CS-AAGCAGACTTGACCTGA (Levesque *et al*, 1995). The PCR amplicons were purified using QIAQuick Gel Extraction kit (Qiagen, Hilden, Germany) and submitted for nucleotide sequencing at Macrogen Inc (Seoul, South Korea). The resulting DNA sequence was analyzed using the BLAST algorithm software available at http://www.ncbi.nlm.nih.gov. Positive controls for the *intI1*, *intI2* and *intI3* genes were *Pseudomonas aeruginosa* P90 (Chuanchuen *et al*, 2007), *Salmonella* Paratyphi B var Java (van Essen-Zandbergen *et al*, 2007) and pAV3.5 (Xu *et al*, 2007), respectively.

Conjugation experiments

Possible conjugal transfer of integrons was investigated using biparental mating (Maniati *et al*, 2007). *P. aeruginosa (n=70)* and *A. baumannii (n=57)* isolates carrying class 1 integrons with resistance gene

Table 1
Antimicrobial susceptibilities of *P. aeruginosa* (*n*=101) and *A. baumannii* (*n*=176).

Antibiotics	*P. aeruginosa*		*A. baumannii*	
	Range of MIC (μg/ml)	% Resistance	Range of MIC (μg/ml)	% Resistance
Amk	8 to >2,048	92.1	<8 to >2,048	88.6
Atm	<1 to >256	96.0	16 to >256	97.2
Car	16 to >2,048	94.1	16 to >2,048	86.4
Cef	4 to >256	81.2	8 to >2,048	90.9
Chp	128 to 512	100.0	64 to 512	99.4
Cip	<0.25 to >256	99.0	0.25 to 256	94.3
Ery	>512	100.0	<2 to >2,048	97.2
Gen	<1 to >256	95.0	<8 to >2,048	94.3
Kan	≥256	100.0	<8 to >2,048	95.6
Neo	64 to >256	100.0	<8 to >2,048	92.0
Pip	<2 to >256	90.1	32 to >1,024	95.5
Spc	≥256	100.0	32 to 2,048	100.0
Str	4 to >256	99.0	<8 to >2,048	97.2
Tet	64 to >256	100.0	<8 to >2,048	94.9
Tri	128 to >256	100.0	16 to >1,024	100.0

Amk, amikacin; Atm, aztreonam; Car, carbenicillin; Cef, ceftaxidime; Chp, chloramphenicol; Cip, ciprofloxacin; Ery, erythromycin; Gen, gentamicin; Kan, kanamycin; Neo, neomycin; Pip, piperacillin; Str, streptomycin; Spc, spectinomycin; Tet, tetracycline; Tri, trimethroprim

cassettes were used as donors. Rifampicin-resistant *E.coli* MG1655 derivatives were recipients. Transconjugants were placed on Luria-bertani (LB) agar (Difco, BD Diagnostic Systems, Detroit, ME) supplemented with 32 μg/ml of rifampicin and one of the following antibiotics: streptomycin (80 μg/ml), gentamicin (100 μg/ml) or trimethroprim (10 μg/ml). Transconjugants were confirmed to be *E. coli* by growth on MacConkey agar (Difco) and transfer of class 1 integrons was confirmed using PCR as described above. The biparental mating procedure was repeated on two separate occasions for each donor-recipient combination yielding no transconjugants. All experiments were carried out at Biosafety Level 2.

RESULTS

Antimicrobial resistance profile

The MIC value and resistance rates of all the isolates tested are shown in Table 1. All *P. aeruginosa* and *A. baumannii* isolates were resistant to at least six antimicrobial agents. All *P. aeruginosa* strains were resistant to chloramphenicol, erythromycin, kanamycin, neomycin, spectinomycin, tetracycline and trimethoprim. Eighty-six isolates (85.2%) were resistant to ceftaxidime, while resistance rates to all other antibiotics tested were above 90%. All *A. baumannii* isolates were resistant to spectinomicin and trimethoprim. Resistance rates to amikacin and carbencillin were 88.6% and 86.4%, respectively. Resistance

rates to all other antibiotics were greater than 90%. The resistance phenotypes of *P. aeruginosa* and *A. baumannii* could be arranged into 14 and 30 patterns, respectively (data not shown). The most common resistance pattern for both *P. aeruginosa* (78.8%) and *A. baumannii* (81.2%) was Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri. Among *P. aeruginosa*, the other resistance patterns present in more than one isolate were: Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Str-Spc-Tet-Tri (2%), Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (2%) and Atm-Chp-Cip-Ery-Kan-Neo-Str-Spc-Tet-Tri (2%). Among *A. baumannii* other resistance patterns were Amk-Atm-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1.7%) and Atm-Cef-Chp-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1.7%).

Class 1 integrons analysis

Ninety-six *P. aeruginosa* isolates (95%) were positive for *intl1*, of which 70 isolates (69.3%) harbored resistance gene cassettes. Twelve integron profiles (IPs) were defined based on the number and the size of the PCR amplicons obtained (Table 2). The most frequently-identified gene cassette array was *aadB-cmlA-aadA1* (37.5%) in IP-XI. Two distinct class 1 integrons containing *aacA4* and *aacA7-cmlA* cassette arrays were found in two *P. aeruginosa* isolates (IP-V).

Intl1 gene amplicons were obtained from 69 *A. baumannii* isolates (39.2%). Fifty-seven isolates (32.4%) carried class 1 integrons with inserted-resistance gene cassettes classified into 13 IPs. Among class 1 integron-carrying isolates, the most common gene cassette combination was *dfrA1-orfC* in IP-I and bla_{VEB-1}-*aadB-arr-2-cmlA-*bla_{OXA-10}-*aadA1* (18.8%) in IP-VI. The presence of a complete *aadA1* gene was additionally tested in all 13 *A. baumannii* strains carrying the gene array *aac(6')I1-aadA1-IS26-tnpA-IS26-aadA1* (IP-VI) and the gene was detected in only two isolates. Coexistence of empty class 1 integrons and gene cassette-containing integrons was found in 8 *A. baumannii* strains. Among these isolates, five strains carried two class 1 integrons (IPs-X and XI) and the others carried three class 1 integrons (IPs-XII and XIII). None of the *P. aeruginosa* or *A. baumannii* strains was found carried *intl2* and *intl3* genes.

Transfer of class 1 integrons

Among *P. aeruginosa* isolates, two class 1 integrons carrying gene cassette arrays *dfrA1-orfC* (IP-IV) and *aadB-cmlA-aadA1* (IP-XI) in the variable regions were conjugally transferred. Five *A. baumannii* strains could horizontally transfer class 1 integrons, including class 1 integrons with the *aac(6')I1-aadA1* array in IP-XI and four empty class 1 integrons in IP-X – XIII. The latter included the empty integrons from two *A. baumannii* isolates in IP-XI and one empty integron each from isolates in IPs-XII and XIII.

DISCUSSION

All *P. aeruginosa* and *A. baumannii* isolates in this study were multidrug resistant; these high resistance rates are in agreement with previous studies (Seward, 1999; Gu *et al*, 2007). These findings were according to our expectations since the pathogens have been infamous for their highly-intrinsic resistance to antibiotics. Resistance to amikacin, piperacillin and ceftzidime is of special concern since these are important drugs of choice for treating *P. aeruginosa* and *A. baumannii* infections. In most cases, infections with these two pathogens did not respond well to anti-

Table 2
Characteristics of class 1 integrons in *P. aeruginosa* (*n*=101) and *A. baumannii* (*n*=176).

IP	Integron size (bp)	Gene cassette[e]	No. of isolates (%)[a]	Resistance pattern
P. aeruginosa				
I	0.8	*aacA4*	3 (3.1)	Atm-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Str-Spc-Tet-Tri (1)
				Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1)
				Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1)
II	1.3	*aadA6*	4 (4.2)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Str-Spc-Tet-Tri (1)
				Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (3)
III	1.3	*aadA6-orfD*	2 (2.1)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (2)
IV	1.3	*dfrA1-orfC*[c]	1 (1.0)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1)
V	0.8, 1.8	*aacA4,* *aacA7-cmlA*	2 (2.1)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (2)
VI	1.8	*bla*$_{IMP-14}$*-aac(6')*	1 (1.0)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1)
VII	2.0	*bla*$_{PSE-1}$*-aadA2*	7 (7.3)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (7)
VIII	2.0	*bla*$_{IMP-15}$*-dhfr-aac(6')*	1 (1.0)	Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Str-Spc-Tet-Tri (1)
IX	2.5	*aadB-bla*$_{OXA-10}$*-aadA1*	7 (7.3)	Amk-Atm-Car-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (5)
				Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (2)
X	2.5	*aadB-arr-2-cmlA-bla*$_{OXA-10}$*-aadA1*	1 (1.0)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1)
XI	3.0	*aadB-cmlA-aadA1*[c]	36 (37.5)	Amk-Chp-Cip-Ery-Gen-Kan-Neo-Str-Spc-Tet-Tri (1)
				Amk-Atm-Car-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (2)
				Amk-Atm-Car-Chp-Cef-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (33)
XII	3.5	*aadB-cmlA-bla*$_{OXA-10}$*-aadA15*	5 (5.2)	Amk-Atm-Car-Chp-Cef-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (5)
A. baumannii				
I	1.2	*dfrA1-orfC*	13 (18.8)	Amk-Atm-Car-Cef-Chp-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (11)
				Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Pip-Str-Spc-Tet-Tri (2)
II	1.6	*aac(6')I1-aadA1*	4 (5.8)	Amk-Atm-Car-Cef-Chp-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (4)
III	1.9	*dfrA12-orfF-aadA2*	1 (1.4)	Amk-Atm-Car-Cef-Chp-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1)
IV	2.5	*aacC1-orfX-orfY-aadA1a*	1 (1.4)	Amk-Atm-Car-Cef-Chp-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1)
V	3.0	*aacC1-orfX -orfX-orfY-aadA1a*	3 (4.3)	Amk-Atm-Car-Cef-Chp-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (3)
VI	2.3	*aac(6')I1-aadA1-IS26-tnpA-IS26-aadA1*	9 (13.0)	Amk-Atm-Car-Cef-Chp-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (9)
VII	5.5	*bla*$_{VEB-1}$*-aadB-arr-2-cmlA-bla*$_{OXA-10}$*-aadA1*	13 (18.8)	Amk-Atm-Car-Cef-Chp-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (13)
VIII	1.9, 2.5	*dfrA12-orfF-aadA2,*	4 (5.8)	Amk-Atm-Car-Cef-Chp-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (4)

IX	1 (1.4)	2.2, 3.0	aacC1-orfX-orfY-aadA1a, aacA4-catB8-aadA1,	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1)
X	2 (2.9)	0.15^b, 1.6	aacC1-orfX-orfX'-orfY-aadA1a	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (2)
XI	3 (4.3)	0.15^d, 3.0	aac(6')I1-aadA1^c	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (3)
XII	2 (2.9)	0.15^d, 1.9, 2.5	aacC1-orfX -orfX'-orfY-aadA1a, dfrA12-orfF-aadA2,	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (2)
XIII	1 (1.4)	0.15^d, 2.2, 3.0	aacC1-orfX-orfY-aadA1a, aacA4-catB8-aadA1, aacC1-orfX-orfX'-orfY-aadA1a	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1)

[a] Total number of isolates used; 96 for *P. aeruginosa* and 69 for *A. baumannii*.
[b] Class 1 integrons without any inserted-gene cassette in variable region.
[c] Capable of horizontal transfer.
[d] Empty integrons conjugally transferred.
[e] Antimicrobial resistance-encoding genes: *aacA4*, *aac(6')* and *aac(6')I1*, amikacin, kanamycin and tobramycin; *aacC1* and *aacA7* , gentamicin; *aadA1*, *aadA2* and *aadA6*, streptomycin and spectinomicin; *aadB*, gentamicin, kanamicin and tobramicin; *bla*$_{PSE-1}$, β-lactams; *bla*$_{OXA-10}$, oxacillin; *bla*$_{IMP-14}$, imipenem and meropenem; *cmlA* and *catB8*, chloramphenicol; *dfrA1* and *dfrA12*, trimethoprim.

biotics. The emergence of resistant *P. aeruginosa* and *A. baumannii* strains reduces the antibiotic treatment options leading to an increased possibility of treatment failure.

In this study, genes conferring resistance to aminoglycosides were frequently found; the most common gene cassettes belonged to the *aad* and *aac* families. The most frequent aminoglycoside-resistance gene cassettes found in class 1 integrons among *P. aeruginosa* was *aadB* and among *A. baumannii* was *aadA1*. The widespread presence of aminoglycoside resistant gene cassettes can be explained by the extensive use of drugs in this class for treatment.

Among *P. aeruginosa*, two Metallo-β-lactamase gene**s** *bla*$_{IMP-14}$ and *bla*$_{IMP-15}$ were identified in combination with *aac(6′)* and *dhfr-aac(6′)*, respectively. Both *bla*$_{IMP-14}$-*aac(6′)* and *bla*$_{IMP-15}$ -*dhfr-aac(6′)* gene cassette arrays were previously described in class 1 integrons in Thailand (GenBank accession no. AY553332 and AY553333, respectively). The *bla*$_{IMP-15}$ gene cassette was previously identified in carbapenem-resistant *P. aeruginosa* strains, but with a different gene cassette array (Garza-Ramos *et al*, 2008). Five gene cassette combinations identified in this study, *aacA7-cmlA*, *aadB-bla*$_{OXA-10}$-*aadA1*, *aadB-arr-2-cmlA-bla*$_{OXA-10}$-*aadA1*, *aadB-cmlA-aadA1* and *aadB-cmlA-bla*$_{OXA-10}$-*aadA15*, have never been previously reported from *P. aeruginosa*, even though all these genes have been demonstrated in other settings and in different orders (Girlich *et al*, 2002; Gu *et al*, 2007). A similar gene cassette array *aadB-arr-2-cmlA-bla*$_{OXA-10}$-*aadA1* was described among *P. aeruginosa* clinical isolates in Thailand (Girlich *et al*, 2002). The difference was the lack of *bla*$_{VEB-like}$ in the array *aadB-arr-2-cmlA-bla*$_{OXA-10}$-*aadA1*, newly discovered in this study. This cassette array could be a result of homologous-recombinational exchange of gene

cassettes between two class 1 integrons or IntI1-mediated site specific recombination (Partridge *et al*, 2002).

The gene cassette arrays identified among *A. baumannii* isolates have been previously found worldwide, for example, the *aacA4-catB-aadA1*, *dfrA12-orfF-aadA2* and *aacC1-orfX-orfY-aadA1a* arrays were demonstrated in clinical isolates from China (Gu *et al*, 2007) and Taiwan (Lee *et al*, 2009). The latter was recently found in class 1 integrons from Australia (Zong *et al*, 2008). The most common gene cassette array identified among *A. baumannii* isolates in this study, bla_{VEB-1}-*aadB-arr-2-cmlA*- bla_{OXA-10}-*aadA1*, was previously characterized among *P. aeruginosa* isolates (Girlich *et al*, 2002). The *dfrA1-orfC* array was found in both *P. aeruginosa* and *A. baumannii*. This gene cassette combination has been previously identified in other bacteria: *Salmonella* spp (Hsu *et al*, 2006) and *Proteus mirabilis* (Boyd *et al*, 2008). The bla_{PSE-1}-*aadA2* array found among *P. aeruginosa* isolates was previously identified in *P. mirabilis* (Boyd *et al*, 2008). The presence of identical gene arrays in different bacterial species or in the same species from different geographical areas suggested the efficient horizontal transfer of class 1 integrons. This notion was confirmed by the presence of class 1 integrons located on conjugative plasmids in this study. Some empty integrons was conjugally transmitted when streptomycin was used as selective pressure, suggesting the expression of other streptomycin-resistant encoding determinants located elsewhere on the same plasmids. This observation highlights the important role of conjugative R-plasmids on the dissemination of resistance among bacteria.

In addition to the *aacC1-orfX-orfY-aadA1a* array, a similar cassette combination with additional *orfX, aacC1-orfX-orfX'*-*orfY-aadA1a* was observed among *A. baumannii* isolates. This gene cassette array has been previously identified; it has been suggested the second copy of *orfX* may be captured by site-specific recombination mechanisms (Turton *et al*, 2005). The *aac(6')/1-aadA1-IS26-tnpA-IS26-aadA1* array was first described among patient isolates from South Korea (Han *et al*, 2008). Since *aadA1* was expected to be inactivated by IS26 insertion, all nine strains carrying this array were resistant to spectinomycin and streptomycin. However, only two isolates contained a complete *aadA1* gene, indicating the existence of unidentified mechanisms encoding for resistance to both aminoglycosides in the other isolates.

Class 1 integrons devoid of gene cassettes were found commonly among *intI1*-positive isolates in this collection (*eg*, 53.6% in *A.baumannii* and 27.1% in *P. aeruginosa*). The empty variable regions in these integrons were available to capture new gene cassette (s) for further horizontal dissemination, even though their sources are still ambiguous.

Class 2 integrons have also been described among both *P. aeruginosa* (Xu *et al*, 2009) and *A. baumannii* (Seward, 1999) but no class 3 integrons have been reported among these pathogens. The absence of class 2 and 3 integrons among the isolates in the present study indicates these two genetic elements did not play a role in antimicrobial resistance among these bacteria. Resistance gene cassettes present in class 1 integrons cannot cover all resistance phenotypes in both pathogens, indicating the existence of other resistance mechanisms not tested. Several resistance mechanisms have been reported among *P. aeruginosa* and *A. baumannii*, such as multidrug efflux systems (Schweizer, 2003; Marchand *et al*, 2004); however, these were

not pursued in this study.

In conclusion, the results confirm the high prevalence of class 1 integrons and their important role in the dissemination of antimicrobial-resistance genes among *P. aeruginosa* and *A. baumannii* isolates in this study. Clinical use of antibiotics may increase selective pressure for MDR strains and for horizontal gene transfer. This could pose a serious threat to the efficacy of antibiotics used for treating infections caused by not only *P. aeruginosa* and *A. baumannii*, but also other clinically-significant pathogens.

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