เอกสารอ้างอิง

Agodi, A., Zarrilli, R., Barchitta, M., Anzaldi, A., Di Popolo, A., Mattaliano, A., Ghiraldi, E. and Travali, S. 2006. Alert surveillance of intensive care unit-acquired *Acinetobacter* infections in a Sicilian hospital. Clin. Microbiol. Infect. 12(3): 241-247.

Boyd, D.A., Shi, X., Hu, Q.H., Ng, L.K., Doublet, B., Cloeckaert, A. and Mulvey, M.R. 2008. *Salmonella* genomic island 1 (SGI1), variant SGI1-I, and new variant SGI1-O in *Proteus mirabilis* clinical and food isolates from China. Antimicrob. Agents Chemother. 52(1): 340-344.

Brizio, A., Conceicao, T., Pimentel, M., Da Silva, G. and Duarte, A. 2006. High-level expression of IMP-5 carbapenemase owing to point mutation in the -35 promoter region of class 1 integron among *Pseudomonas aeruginosa* clinical isolates. Int. J. Antimicrob. Agents. 27(1): 27-31.

Cheng, X., Wang, P., Wang, Y., Zhang, H., Tao, C., Yang, W., Liu, M. and Jia, W. 2008. Identification and distribution of the clinical isolates of imipenem-resistant *Pseudomonas aeruginosa* carrying metallo-*beta*-lactamase and/or class 1 integron genes. J. Huazhong Univ. Sci. Technolog. Med. Sci. 28(3): 235-238.

Fluit, A.C. and Schmitz, F.J. 2004. Resistance integrons and super-integrons. Clin. Microbiol. Infect. 10(4): 272-288.

Girlich, D., Naas, T., Leelaporn, A., Poirel, L., Fennewald, M. and Nordmann, P. 2002. Nosocomial spread of the integron-located veb-1-like cassette encoding an extended-pectrum beta-lactamase in *Pseudomonas aeruginosa* in Thailand. Clin. Infect. Dis. 34(5): 603-611.

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

Hall, R.M. and Stokes, H.W. 1993. Integrons: novel DNA elements which capture genes by site-specific recombination. Genetica. 90(2-3): 115-132.

Han, H.L., Jang, S.J., Park, G., Kook, J.K., Shin, J.H., Shin, S.H., Kim, D.M., Cheon, J.S., Moon, D.S. and Park, Y.J. 2008. Identification of an atypical integron carrying an IS26-disrupted aadA1 gene cassette in *Acinetobacter baumannii*. Int. J. Antimicrob. Agents. 32(2): 165-169.

Hsu, S.C., Chiu, T.H., Pang, J.C., Hsuan-Yuan, C.H., Chang, G.N. and Tsen, H.Y. 2006. Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. Int. J. Antimicrob. Agents. 27(5): 383-391.

Khan, A.A., Ponce, E., Nawaz, M.S., Cheng, C.M., Khan, J.A. and West, C.S. 2009. Identification and characterization of Class 1 integron resistance gene cassettes among *Salmonella* strains isolated from imported seafood. Appl. Environ. Microbiol. 75(4): 1192-1196.

Kim, J.Y., Park, Y.J., Kwon, H.J., Han, K., Kang, M.W. and Woo, G.J. 2008. Occurrence and mechanisms of amikacin resistance and its association with beta-lactamases in *Pseudomonas aeruginosa*: a Korean nationwide study. J. Antimicrob. Chemother. 62(3): 479-483.

Kraniotaki, E., Manganelli, R., Platsouka, E., Grossato, A., Paniara, O. and Palu, G. 2006. Molecular investigation of an outbreak of multidrug-resistant *Acinetobacter baumannii*, with characterisation of class 1 integrons. Int. J. Antimicrob. Agents. 28(3): 193-199.

Labuschagne Cde, J., Weldhagen, G.F., Ehlers, M.M. and Dove, M.G. 2008. Emergence of class 1 integron-associated GES-5 and GES-5-like extended-spectrum beta-lactamases in clinical isolates of *Pseudomonas aeruginosa* in South Africa. Int. J. Antimicrob. Agents. 31(6): 527-530.

Lee, Y.T., Huang, L.Y., Chen, T.L., Siu, L.K., Fung, C.P., Cho, W.L., Yu, K.W. and Liu, C.Y. 2009. Gene cassette arrays, antibiotic susceptibilities, and clinical characteristics of *Acinetobacter baumannii* bacteremic strains harboring class 1 integrons. J. Microbiol. Immunol. Infect. 42(3): 210-219.

Liu, S.Y., Lin, J.Y., Chu, C., Su, L.H., Lin, T.Y. and Chiu, C.H. 2006. Integron-associated imipenem resistance in *Acinetobacter baumannii* isolated from a regional hospital in Taiwan. Int. J. Antimicrob. Agents. 27(1): 81-84.

Miko, A., Pries, K., Schroeter, A. and Helmuth, R. 2005. Molecular mechanisms of resistance in multidrug-resistant serovars of *Salmonella enterica* isolated from foods in Germany. J. Antimicrob. Chemother. 56(6): 1025-1033.

Naas, T., Aubert, D., Lambert, T. and Nordmann, P. 2006. Complex genetic structures with repeated elements, a *sul*-type class 1 integron, and the *blaVEB* extended-spectrum *beta*-lactamase gene. Antimicrob. Agents Chemother. 50(5): 1745-1752.

Partridge, S.R., Tsafnat, G., Coiera, E. and Iredell, J.R. 2009. Gene cassettes and cassette arrays in mobile resistance integrons. FEMS Microbiol. Rev. 33(4): 757-784.

Ramirez, M.S., Quiroga, C. and Centron, D. 2005. Novel rearrangement of a class 2 integron in two non-epidemiologically related isolates of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 49(12): 5179-5181.

Sevillano, E., Valderrey, C., Canduela, M.J., Umaran, A., Calvo, F. and Gallego, L. 2006. Resistance to antibiotics in clinical isolates of *Pseudomonas aeruginosa*. Pathol. Biol. (Paris). 54(8-9): 493-497.

Shahcheraghi, F., Badmasti, F. and Feizabadi, M.M. 2010. Molecular characterization of class 1 integrons in MDR *Pseudomonas aeruginosa* isolated from clinical settings in Iran, Tehran. FEMS Immunol. Med. Microbiol. 58(3): 421-425.

Sunde, M. 2005. Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. J. Antimicrob. Chemother. 56(6): 1019-1024.

Syrmis, M., Bell, S., Bye, P., Coulter, C., Harbour, C., Iredell, J., Kidd, T., O'Carroll, M., Rose, B., Wainwright, C., Sloots, T. and Nissen, M. 2008. High prevalence of a class 1 integron-associated *aadB* gene cassette in *Pseudomonas aeruginosa* isolates from an Australian cystic fibrosis patient population. Pathology. 40(5): 524-525.

Valenzuela, J.K., Thomas, L., Partridge, S.R., van der Reijden, T., Dijkshoorn, L. and Iredell, J. 2007. Horizontal gene transfer in a polyclonal outbreak of carbapenem-resistant *Acinetobacter baumannii*. J. Clin. Microbiol. 45(2): 453-460.

van Essen-Zandbergen, A., Smith, H., Veldman, K. and Mevius, D. 2007. Occurrence and characteristics of class 1, 2 and 3 integrons in *Escherichia coli*, *Salmonella* and *Campylobacter* spp. in the Netherlands. J. Antimicrob. Chemother. 59(4): 746-750.

Vo, A.T., van Duijkeren, E., Fluit, A.C., Wannet, W.J., Verbruggen, A.J., Maas, H.M. and Gaastra, W. 2006. Antibiotic resistance, integrons and *Salmonella* genomic island 1 among non-typhoidal *Salmonella* serovars in The Netherlands. Int. J. Antimicrob. Agents. 28(3): 172-179.

Xu, X., Kong, F., Cheng, X., Yan, B., Du, X., Gai, J., Ai, H., Shi, L. and Iredell, J. 2008. Integron gene cassettes in *Acinetobacter* spp. strains from South China. Int. J. Antimicrob. Agents. 32(5): 441-445.

ภาคผนวก

อาหารเลี้ยงเชื้อ

1. Muller Hinton Agar (MHA) (Difco™, MD, USA)

- Beef Extract Powder	2.0	g
- Acid Digest of Casien	17.5	g
- Starch	1.5	g
- Agar	17.0	g

2. Luria-Bertani Agar (LB) (Difco™, MD, USA)

- Trptone	10.0	g
- Yeast Extract	5.0	g
- Sodium chloride	5.0	g
- Agar	15.0	g

ยาปฏิชีวนะและสารเคมีอื่น ๆ

1. 50X TAE (Tris-Acetate buffer) ใน 1,000 ml ประกอบด้วย

- Tris	242.0	g
- Acetic acid	57.1	g
- 0.5M EDTA pH 8.0	100.0	ml

2. 0.5 M EDTA, pH 8.0 ใน 1,000 ml ประกอบด้วย

- Disodium ethylene diamine tetraacetate. $2H_2O$	186.1	g
- Distilled deionized water	800.0	ml
- Adjust pH to 8.0		

3. 1 M Tris HCl, pH 8.0 ใน 1,000 ml ประกอบด้วย

- Tris (ultrapure)	121.1	g
- Distilled deionized water	800.0	ml
- Adjust pH to 8.0 by adding conc. HCL	42.0 ml	

4. การเตรียมยาปฏิชีวนะ

Antibiotics	Solvents	Concentrations range (µg/ml)
Carbenicillin	Water	8, 16, 32, 64, 128, 256, 512, 1024, 2048
Piperacillin	Water	4, 8, 16, 32, 64, 128, 256, 512
Ceftaxidime	Water	1, 2, 4, 8, 16, 32, 64, 128, 256
Aztreonam	Saturated sodium bicarbonate	1, 2, 4, 8, 16, 32, 64, 128, 256
Amikacin	Water	4, 8, 16, 32, 64, 128, 256, 512
Gentamicin	Water	1, 2, 4, 8, 16, 32, 64, 128, 256
Kanamycin	Water	1, 2, 4, 8, 16, 32, 64, 128, 256
Neomycin	Water	1, 2, 4, 8, 16, 32, 64, 128, 256
Streptomycin	Water	1, 2, 4, 8, 16, 32, 64, 128, 256
Spectinomycin	Water	1, 2, 4, 8, 16, 32, 64, 128, 256
Ciprofloxacin	0.1N NaOH	0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256
Tetracycline	70% ethanol	1, 2, 4, 8, 16, 32, 64, 128, 256
Erythromycin	Water	1, 2, 4, 8, 16, 32, 64, 128, 256
Chloramphenicol	95% ethanol	1, 2, 4, 8, 16, 32, 64, 128, 256, 512
Trimethiprim	dimethylacetamide	1, 2, 4, 8, 16, 32, 64, 128, 256

Outputs ที่ได้จากผลงานวิจัยครั้งนี้

Kanchana Poonsuk, Chanwit Tribuddharat and Rungtip Chuanchuen. Class 1 integrons in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated human clinical cases. Southeast Asian Journal of Public Health and Tropical Medicine **(submitted on 27/1/2011)**

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

Kanchana Poonsuk[1] Chanwit Tribuddharat[2] and Rungtip Chuanchuen[1], *

[1]*Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330 Thailand*

[2]*Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University 10700 Thailand*

Correspondence: Dr Rungtip Chuanchuen, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok 10300, Thailand.

Tel: (66) (0) 218-9578; Fax: 66 (0) 218-9577

E-mail:rchuanchuen@yahoo.com

Running head: Characterization of class 1 integrons

Key Words *Acinetobacter baumannii*; class 1 integrons; multidrug resistance; *Pseudomonas aeruginosa*

Abstract. A hundred and one *Pseudomonas aeruginosa* isolates and 176 *Acinetobacter baumannii* isolates were characterized for integrons. Among these isolates, class 1 integrons with resistance gene cassette were detected in 69.3% *P. aeruginosa* and 31.8% *A. baumannii* but class 2 and 3 integrons were not found. Five novel gene cassette arrays were identified in *P. aeruginosa*, including *aacA7-cmlA*, *aadB-*$bla_{\text{OXA-10}}$*-aadA1*, *aadB-arr-2-cmlA-*$bla_{\text{OXA-10}}$*-aadA1*, *aadB-cmlA-aadA1* and *aadB-cmlA-*$bla_{\text{OXA-10}}$*-aadA15*. Most integrons found in *A. baumannii* were worldwide distributed. Horizontal transfer of some class 1 integrons was detected in both *P. aeruginosa* (2/70) and *A. baumannii* (5/57).

INTRODUCTION

Pseudomonas aeruginosa and *Acinetobacter baumannii* have been recognized as predominant nosocomial pathogens, of which clinical significance is due to their multidrug-resistant (MDR) phenotypes leading to therapeutic failure. 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 that are able to integrate and mobilize the 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 was most prevalent among bacteria including *P. aeruginosa* and *A. baumannii* (Gu *et al*, 2007). Of particular concern is class 1 integrons are frequently located on plasmids and transposons that have the ability to undergo horizontal transmission and contribute to rapid dissemination of antibiotic resistance genes among bacterial isolates not limited to *P. aeruginosa* and *A. baumannii* (Fluit and Schmitz, 1999).

To date, resistance to various antibiotics is common among *P. aeruginosa* and *A. baumannii* isolates in many parts of the world, including Thailand. However, there is currently a relative paucity of data on integron-associated gene cassettes among MDR *P. aeruginosa* and *A. baumannii* strains, particularly in most 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.

สำนักงานคณะกรรมการวิจัยแห่งชาติ
ห้องสมุดงานวิจัย

MATERIALS AND METHODS

Bacterial isolates and antimicrobial susceptibility testing.

A hundred and one *P. aeruginosa* isolates and 176 *A. baumannii* isolates were randomly selected from the stock of Siriraj hospital, Bangkok Thailand. All the strains were obtained from a variety of clinical specimens collected during 2001-2008. All bacterial strains were identified by using the VITEK GNI card (bioMérieux Vitek, Inc., Hazelwood, Mo.) and the API 20NE system (bioMérieux, Inc.). Only one colony was collected from each positive patient. However, the genetic relatedness of these isolates was not tested. All the isolates were tested for their minimum inhibitory concentrations (MICs) of 15 antimicrobial agents including 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 the CLSI guidelines (CLSI) (NCCLS, 2002). The *P. aeruginosa* ATCC27853 type strain was used as a control. Multidrug resistance was defined as resistant 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 the integrase genes of the *intI1*and *intI2* and *intI3* genes 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 CS-PCR using a 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). The *P. aeruginosa (n=70)* and *A. baumannii (n=57)* isolates carrying class 1 integrons with resistance gene cassettes were used as donors. Rifampicin-resistant *E.coli* MG1655 derivatives were recipients. Transconjugants were selected using resistance phenotypes for streptomycin (80 μg/ml), gentamycin (100 μg/ml), and trimethroprim (10 μg/ml). Transfer of class 1 integrons was confirmed using PCR as described above.

RESULTS

Antimicrobial resistance profile

All the *P. aeruginosa* and *A. baumannii* isolates were resistant to at least six antimicrobial agents. Among the *P. aeruginosa* strains, all 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 the *A. baumannii* isolates were absolutely 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 higher than 90%. The resistance phenotypes of *P. aeruginosa* and *A. baumannii* could be arranged into 14 and 30 patterns, respectively. The most common resistance pattern in 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.

Class 1 integrons analysis

Ninety-six *P. aeruginosa* isolates (95%) were positive to *intI1*, 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 1). The most frequently-identified gene cassette array was *aadB-cmlA-aadA1* (37.5%) in IP-XI. Two distinct class 1 integrons containing the *aacA4* and *aacA7-cmlA* cassette arrays were found in two *P. aeruginosa* isolates (IP-V).

The *intI1* gene amplicons were obtained from 69 *A. baumannii* isolates (39.2%). Fifty-six isolates (31.8%) carried class 1integrons with inserted-resistance gene cassettes that were classified into 13 IPs. Of the class 1 integrons-carrying isolates, the most common gene cassette combination was $bla_{\text{VEB-1}}$-*aadB-arr-2-cmlA*-$bla_{\text{OXA-10}}$-*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 harbored three class 1 integrons (IPs-XII and XIII).

None of the *P. aeruginosa* and *A. baumannii* strains was found to possess class 2 and 3 integrons.

Transfer of class 1 integrons.

Among the *P. aeruginosa* isolates, two class 1 integrons carrying the 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-X and four empty class 1 integrons in IP-XI – XIII. The latter included the empty integrons from two *A. baumannii* isolates in IP-XI and one of each empty integrons from the isolate in IPs-XII and XIII.

DISCUSSION

All *P. aeruginosa* and *A. baumannii* isolates in this study are multidrug resistant, in agreement with previous studies (Seward, 1999; Gu *et al*, 2007). This was not far from our expectation since the pathogens have been infamous for their highlyintrinsic resistance to antibiotics. Resistance to amikacin, piperacillin and ceftzidime are of special concerns since they are important drugs of choice for treatment of *P. aeruginosa* and *A. baumannii* infections. In most cases, infections with these two pathogens do not respond well to antibiotic treatment. Therefore, the emergence of such resistant *P. aeruginosa* and *A. baumannii* strains may diminish the alternative antibiotics leading to the increased possibility of treatment failure.

In this study, genes conferring resistance to aminoglycosides were frequently found, of which the most common gene cassettes belong to the *aad* and *aac* families. The most frequent aminoglycoside-resistance gene cassettes found in class 1 integrons from *P. aeruginosa* was *aadB*, while that in *A. baumannii* was *aadA1*. The widespread of aminoglycosides resistance gene cassettes can be explained by the extensive use of drugs in this class for infection therapy.

In *P. aeruginosa*, two Metallo-β-lactamase genes *bla*$_{\text{IMP-14}}$ and *bla*$_{\text{IMP-15}}$ were identified in combination with different genes, *aac(6')* and *dhfr-aac(6')*, respectively. Both *bla*$_{\text{IMP-14}}$-*aac(6')* and *bla*$_{\text{IMP-15}}$ -*dhfr-aac(6')* gene cassette arrays were previously described in class 1 integrons in Thailand (GenBank accession no.AY553332 and AY553333, respectively). The *bla*$_{\text{IMP-15}}$ 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, including *aacA7-cmlA* , *aadB-bla*$_{\text{OXA-10}}$-*aadA1*, *aadB-arr-2-cmlA-bla*$_{\text{OXA-10}}$-*aadA1*, *aadB-cmlA-aadA1* and *aadB-cmlA-bla*$_{\text{OXA-10}}$-*aadA15* have never been previously reported in *P. aeruginosa*, even though all these genes have been demonstrated in other settings and different orders (Girlich *et al*, 2002; Gu *et al*, 2007). A similar gene cassette array *aadB-arr-2-cmlA-bla*$_{\text{OXA-10}}$-*aadA1* was described in *P. aeruginosa* clinical isolates in Thailand (Girlich *et al*, 2002). The difference was the lack of *bla*$_{VEB\text{-}like}$ in the array *aadB-arr-2-cmlA-bla*$_{OXA\text{-}10}$*-aadA1* newly determined 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 in *A. baumannii* have been previously found worldwide, for example, the *aacA4-catB-aadA1*, *dfrA12-orfF-aadA2* and *aacC1-orfX-orfY-aadA1a* arrays were demonstrated in the clinical isolates in 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 in *A. baumannii* in this study, *bla*$_{VEB\text{-}1}$*-aadB-arr-2-cmlA- bla*$_{OXA\text{-}10}$*-aadA1*, was previously characterized in *P. aeruginosa* (Girlich *et al*, 2002). Concurrently, the *dfrA1-orfC* array was found in both *P. aeruginosa* and *A. baumannii*. This gene cassette combination was previously identified in other bacteria e.g. *Salmonella* (Hsu *et al*, 2006) and *Proteus mirabilis* (Boyd *et al*, 2008). In addition, the *bla*$_{PSE\text{-}1}$*-aadA2* array found in *P. aeruginosa* was previously identified in *P. mirabilis* (Boyd *et al*, 2008). The presence of the 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. In addition, some empty integrons was conjugally transmitted when streptomycin was used as a selective pressure, suggesting the expression of other streptomycin-resistance encoding determinants located elsewhere on the same plasmids. This observation highlights yet again the important role of conjugative R-plasmids on 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 in *A. baumannii*. This gene cassette array was previously identified and it was suggested that 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 in the patient isolates from South Korea (Han *et al*, 2008). As *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 in resistance to both aminoglycosides in the other isolates.

Class 1 integrons devoid of gene cassettes were found at high rate among the isolates in this collection. The empty variable regions in these integrons are available to capture the new coming gene cassette (s) that may be readily for further horizontal dissemination even though their sources are still ambiguous,.

In addition to class 1 integrons, class 2 integrons has been described in both *P. aeruginosa* (Xu *et al*, 2009) and *A. baumannii* (Seward, 1999) but no class 3 integrons has been reported in these pathogens. The absence of class 2 and 3 integrons among all the isolates in the present study indicated that these two genetic elements did not play a role in antimicrobial resistance among the bacterial population. Moreover, the resistance gene cassettes present in class 1 integrons cannot cover all resistance phenotypes of both pathogens, indicating the existence of other resistance mechanisms that were not tested. Several resistance mechanisms have been reported in *P. aeruginosa* and *A. baumanni* e.g. multidrug efflux systems (Schweizer, 2003; Marchand *et al*, 2004); however, they were not pursued in this study.

In conclusion, the results confirm the high prevalence of class 1 integrons and their important role in the uptake and dissemination of antimicrobial-resistance genes among *P. aeruginosa* and *A. baumannii* from patients. Clinical use of antibiotics may increase a selective pressure for the MDR strains and for horizontal transmission. This could be 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.

ACKNOWLEDGEMENT

We thank Dr. Sirintip Khemtong, Faculty of Veterinary Science, Chulalongkorn University for technical assistance. This work was financially supported by Rachadapisaksompoch grant in the fiscal year 2009, Chulalongkorn University. KP is the recipient of the Royal Golden Jubilee Ph.D. program PHD/0014/2552.

REFFERENCES

Boyd DA, Shi X, Hu QH, *et al. Salmonella* genomic island 1 (SGI1), variant SGI1-I, and new variant SGI1-O in *Proteus mirabilis* clinical and food isolates from China. *Antimicrob Agents Chemother* 2008; 52: 340-4.

Chuanchuen R, Khemtong S, Padungtod P. Occurrence of *qacE/qacED1* genes and their correlation with class 1 integrons in *Salmonella enterica* isolates from poultry and swine. *Southeast Asian J Trop Med Public Health* 2007; 38: 855-62.

Ekkapobyotin C, Padungtod P, Chuanchuen R. Antimicrobial resistance of *Campylobacter coli* isolates from swine. *Int J Food Microbiol* 2008; 128: 325-8.

Fluit AC, Schmitz FJ. Class 1 integrons, gene cassettes, mobility, and epidemiology. *Eur J Clin Microbiol Infect Dis* 1999; 18: 761-70.

Garza-Ramos U, Morfin-Otero R, Sader HS, *et al.* Metallo-beta-lactamase gene $bla_{\text{(IMP-15)}}$ in a class 1 integron, In95, from *Pseudomonas aeruginosa* clinical isolates from a hospital in Mexico. *Antimicrob Agents Chemother* 2008; 52: 2943-6.

Girlich D, Naas T, Leelaporn A, Poirel L, Fennewald M, Nordmann P. Nosocomial spread of the integron-located veb-1-like cassette encoding an extended-pectrum beta-lactamase in *Pseudomonas aeruginosa* in Thailand. *Clin Infect Dis* 2002; 34: 603-11.

Gu B, Tong M, Zhao W, *et al.* Prevalence and characterization of class I integrons among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates from patients in Nanjing, China. *J Clin Microbiol* 2007; 45: 241-3.

Han HL, Jang SJ, Park G, *et al.* Identification of an atypical integron carrying an IS26-disrupted *aadA1* gene cassette in *Acinetobacter baumannii. Int J Antimicrob Agents* 2008; 32: 165-9.

Hsu SC, Chiu TH, Pang JC, Hsuan-Yuan CH, Chang GN, Tsen HY. Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. *Int J Antimicrob Agents* 2006; 27: 383-91.

Khemtong S, Chuanchuen R. Class 1 Integrons and *Salmonella* Genomic Island 1 among *Salmonella enterica* Isolated from Poultry and Swine. *Microbe Drug Resist* 2008; 14: 65-70.

Lee YT, Huang LY, Chen TL, *et al.* Gene cassette arrays, antibiotic susceptibilities, and clinical characteristics of *Acinetobacter baumannii* bacteremic strains harboring class 1 integrons. *J Microbiol Immunol Infect* 2009; 42: 210-9.

Levesque C, Piche L, Larose C, Roy PH. PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother* 1995; 39: 185-91.

Maniati M, Ikonomidis A, Mantzana P, Daponte A, Maniatis AN, Pournaras S. A highly carbapenem-resistant *Pseudomonas aeruginosa* isolate with a novel blaVIM-4/blaP1b integron overexpresses two efflux pumps and lacks OprD. *J Antimicrob Chemother* 2007; 60: 132-5.

Marchand I, Damier-Piolle L, Courvalin P, Lambert T. Expression of the RND-type efflux pump AdeABC in *Acinetobacter baumannii* is regulated by the AdeRS two-component system. *Antimicrob Agents Chemother* 2004; 48: 3298-304.

NCCLS. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-Second Edition. NCCLS document M31-A2. PA, USA: NCCLS; 2002.

Partridge SR, Collis CM, Hall RM. Class 1 integron containing a new gene cassette, *aadA10*, associated with Tn1404 from R151. *Antimicrob Agents Chemother* 2002; 46: 2400-8.

Rosser SJ, Young HK. Identification and characterization of class 1 integrons in bacteria from an aquatic environment. *J Antimicrob Chemother* 1999; 44: 11-8.

Schweizer HP. Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeruginosa* and related bacteria: unanswered questions. *Genet Mol Res* 2003; 2: 48-62.

Seward RJ. Detection of integrons in worldwide nosocomial isolates of *Acinetobacter* spp. *Clin Microbiol Infect* 1999; 5: 308-18.

Turton JF, Kaufmann ME, Glover J, *et al.* Detection and typing of integrons in epidemic strains of *Acinetobacter baumannii* found in the United Kingdom. *J Clin Microbiol* 2005; 43: 3074-82.

van Essen-Zandbergen A, Smith H, Veldman K, Mevius D. Occurrence and characteristics of class 1, 2 and 3 integrons in *Escherichia coli*, *Salmonella* and *Campylobacter* spp. in the Netherlands. *J Antimicrob Chemother* 2007; 59: 746-50.

Xu H, Davies J, Miao V. Molecular characterization of class 3 integrons from *Delftia* spp. *J Bacteriol* 2007; 189: 6276-83.

Xu Z, Li L, Shirtliff ME, Alam MJ, Yamasaki S, Shi L. Occurrence and characteristics of class 1 and 2 integrons in *Pseudomonas aeruginosa* isolates from patients in southern China. *J Clin Microbiol* 2009; 47: 230-4.

Zong Z, Lu X, Valenzuela JK, Partridge SR, Iredell J. An outbreak of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemase in western China. *Int J Antimicrob Agents* 2008; 31: 50-4.

สำนักงานคณะกรรมการวิจัยแห่งชาติ ห้องสมุดงานวิจัย

Table 1 Characteristics of class 1 integrons in the *P. aeruginosa* (*n=101*) and *A. baumannii* strains (*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-Cip-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-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (4)
III	1.9	*dfrA12-orfF-aadA2*	1(1.4)	Amk-Atm-Car-Cef-Chp-Cip-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-Cip-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-Cip-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-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (9)
VII	5.5	bla_{VEB-1}-*aadB-arr-2-cmlA*-bla_{OXA-10}-	13 (18.8)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (13)
VIII	1.9, 2.5	*aadA1* *dfrA12-orfF-aadA2*,	4 (5.8)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (4)
IX	2.2, 3.0	*aacC1-orfX-orfY-aadA1a* *aacA4-catB8-aadA1*,	1(1.4)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (1)
X	0.15[b], 1.6	*aacC1-orfX-orfX'-orfY-aadA1a*	2(2.9)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (2)
XI	0.15[d], 3.0	*aac(6')I1-aadA1*[c]	3(4.3)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (3)
XII	0.15[d], 1.9, 2.5	*aacC1-orfX -orfX'-orfY-aadA1a* *dfrA12-orfF-aadA2*,	2(2.9)	Amk-Atm-Car-Cef-Chp-Cip-Ery-Gen-Kan-Neo-Pip-Str-Spc-Tet-Tri (2)
XIII	0.15[d], 2.2, 3.0	*aacC1-orfX-orfY-aadA1a* *aacA4-catB8-aadA1*, *aacC1-orfX-orfX'-orfY-aadA1a*	1(1.4)	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, aacA7* and *aac(3")-Ia,* 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; *dhfr1* and *dfrA12*, trimethoprim