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RESEARCH ARTICLE

Class 1 integron element in Thai *Acinetobacter baumannii* reveals a linkage to the European clone I

Thapa B,[1] Tribuddharat C,[2]* Srifuengfung S,[2] Dhiraputra C[2]

[1]Genesis Laboratory and Research, Kathmandu Nepal, [2]Department of Microbiology, Faculty of medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

*__Correspondence to__: Dr. Chanwit Tribuddharat, Department of Microbiology, Faculty of medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, email: sictb@mahidol.ac.th, Tel. No.: (+066)-816326331

ABSTRACT

BACKGROUND: Class 1 integron element is innate to most of the multidrug resistant *Acinetobacter baumannii* and its spread is common among international clones worldwide. The aim of this study was to document the presence of bla_{VEB-1} harboring class 1 integron element and its gene cassettes in Thai *A. baumannii* in relation to *A. baumannii* European clone I, AYE strain.

MATERIALS AND METHODS: Thirty seven carbapenem resistant *A. baumannii* isolates identified in routine microbiology laboratory of Siriraj Hospital, Bangkok were studied. The dot blot hybridization was performed to detect class 1 integron element integrase gene. PCR was used to amplify bla_{VEB-1}, *arr-2*, *cmlA*, bla_{OXA-10} resistance cassettes, and variable region of class 1 integron element. bla_{VEB-1} gene was localized by southern blot hybridization.

RESULTS: The prevalence of class 1 integron element was 86.48% in the isolates studied. The bla_{VEB-1} was present in 7 isolates however the location of bla_{VEB-1} gene was different in different isolate. Four isolates (Ab03-168, Ab04-28, Ab08-20, and Ab08-22) harbored calss 1 integron element variable region sized 5.5 kb as described in strain AYE. However, bla_{VEB-1} was only amplified from Ab03-168. The cassette organization in this isolate was 5'CS-aadB-bla_{VEB-1}-*arr-2*-*cmlA*-bla_{OXA-10}-aadA1-3'CS. The class 1 integron element similar to the element identified in genomic resistance island, AbaRI of European clone I, AYE was identified in Thai *A. baumannii*.

CONCLUSIONS: bla_{VEB-1} harboring class 1 integron element with minor cassette variation was identified in Thai *A. baumanni* isolate which might suggest the spread of this resistant cassette or the spread of the European clone I in Thailand. Monitoring of the global spread of multi-resistant *A. baumannii* is mandatory to control the spread of resistant genes and this multi-resistant pathogen.

KEY WORDS: Class 1 integron element, *intI1*, bla_{VEB-1}, International clones, *A. baumannii*

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INTRODUCTION

A. baumannii is an emerging nosocomial pathogen inflicting debilitating patients in intensive care units. The increasing resistance to most of the sophisticated antibiotics (carbapenems, tigecycline, and colistin) used in clinical practice or spread of multi-resistant international clones of *A. baumannii* is responsible for high morbidity and mortality (30-70%).[1-3] The genetic basis of resistance to these antibiotics have been contributed by innate resistant determinants, acquisition of resistant genes, integron elements to genomic resistance islands (GRI), and mutation in drug target sites.[4] Integron element carries resistant gene cassettes which is a mobile genetic element.[5] Among three classes of integron elements, class 1 integron element is the commonest in Gram negative bacteria including *A. baumannii*.

Integron element can be considered as a minigenomic resistant island that carries single to multiple drug resistant cassettes and isolates possessing it correlates with resistant phenotype.[1] Resistance gene cassettes to aminoglycoside (*aadB*, *aac3*, *aadA1*, and *aacA4*), β-lactamas (*bla*$_{VEB-1}$,[1] *bla*$_{IMP-1}$, *bla*$_{VIM}$, and *bla*$_{SIM}$,[6] *bla*$_{OXA-2}$, *bla*$_{OXA-3}$, *bla*$_{OXA-10}$, *bla*$_{OXA-21}$, *bla*$_{OXA-20}$, and, *bla*$_{OXA-37}$,[1,7] sulphonamides (*dfr*1),[1] chloramphenicol (*cmlA*, *cmlA5*, and *catB*), and rifampicin (*arr-2*)[1,8,9] are class 1 integron borne and have been described in international clones of *A. baumannii*. The largest GRI, AbaR1 has been described in French strain AYE, is 86 Kb, and harbors three class 1 integron elements.[1] The largest integron element in AbaR1 is a composite class 1 integron element consisting of *bla*$_{VEB-1}$, *aadB*, *arr-2*, *cmlA, bla*$_{OXA-10}$, and *aadA1* cassettes.[1]

The study of class 1 integron elements and these resistance gene cassettes in *Enterobacteriaceae* and non-*Enterobacteriaceae* is common in this hospital and abroad. However, the presence of *bla*$_{VEB-1}$ harboring class 1 integron element, its cassette organization in relation to *bla*$_{VEB-1}$ harboring class 1 integron element of *A. baumannii,* European clone I, AYE strain has not been studied. Here, we studied the presence of *bla*$_{VEB-1}$ harboring class 1 integron element and its gene cassettes organization in Thai *A. baumannii* in relation to European clone I, *A. baumannii* AYE strain.

MATERIALS AND METHODS

Bacterial strains, DNA extraction, and controls: Thirty seven clinical isolates of carbapenem resistant-*Acinetobacter baumannii* (CRAB) from Siriraj Hospital, Bangkok, Thailand were studied.[11] Chromosomal DNA was extracted using the Genomic DNA purification kit (Puregene, Minneapolis, Minnesota, USA) following the manufacturer's protocol. The pCTF202, a plasmid construct carrying an incomplete class 1 integron element (5'CS- *bla*$_{VEB-1}$- *arr-2-cmlA*-truncated *bla*$_{OXA-10}$)[8] and *P. aeruginosa* strain, 58 carrying a complete class 1 integron element (5'CS -*bla*$_{VEB-1}$-*arr-2-cmlA*-*bla*$_{OXA-10}$-*aadA1*-3'CS) were used as a positive control in PCR amplification, dot blot hybridization, and cassette characterization of *bla*$_{VEB-1}$ containing class 1 integron element.

Polymerase chain reaction (PCR): PCR amplification was done in 50 µl reaction volumes containing 0.2 µM of each primer (Table 1), 2mM of each dNTP (FINZYMES), 1 µl (100 ng) of genomic DNA extract, 1 U of DNA polymerase (FINZYMES), and 5 µl of supplied PCR buffer. Amplification was done using GeneAmp PCR system 2400 (PERKIN ELMER) using the following profile: initial denaturation at 95˚C for 5 minutes, 30 cycles of denaturation at 95˚C for 1 minute, annealing at 55˚C for 1 minute, and extension at 72˚C for 1 minute/1000 nucleotides, and a final extension at 72˚C for 7 minutes. Amplified products were detected by agarose gel electrophoresis in 1% Tris-Acetate-EDTA (TAE) agarose (Research organics, inc. USA) stained with ethedium bromide.

Hybridization for *intI1* detection and *bla*$_{VEB-1}$ localization: Class 1 integron integrase gene (*intI1*) and *bla*$_{VEB-1}$ was amplified from the plasmid pCTF202 using the primer sets, IntI1F/IntI1R and VEB1F/VEB1R, respectively. The 471bp and 642bp amplicons generated were purified using the PCR product purification kit (PCR clean up kit). Both of these DNA were labeled with the random primer labeling kit (Roche, USA). Dot blot and southern blot hybridization were performed on these chromosomal DNA as per the manufacturer's protocol (Amersham, UK). *Eco*RI restriction enzyme was used for southern blot hybridization. Plasmids pCTF202 and pUC19 were used as positive and negative controls, respectively. Ethical clearance was taken from the institution where the study was conducted.

RESULTS

Detection of *IntI1* and resistant cassettes

The dot blot hybridization using 471 bp amplicon of *intI1* gene as a probe was able to detect *intI1*in 30 isolates (81.08%). The dot blot negative isolates were Ab08-5, -6, -07, -24, -25, -26, and -28 (Fig.1).

Table 1. Primers used in this study

Primer Name	Target	Amplicon (bp)	Primer sequence (5'-3')
IntI1F	*IntI1*	471	AAGGATCGGGCCTTGATGTT
IntI1R			CAGCGCATCAAGCGGTGAGC
5'CS	Variable region of class 1 integron element	variable	GGCATCCAAGCAGCAAG
3'CS			AAGCAGACTTGACCTGA
VEB1F	$bBla_{VEB-1}$	642	CCACTTCCATTTCCCGATGC
VEB1R			GGACTCTGCAACAAATACGC
CMLAF2	*cmlA*	455	ACTAATGATGGCAGGCAAG
CMLAR2			AAGACAGACCGAGCACGACT
ARR-2F	*arr-2*	417	CATTTCGAGGACGGTCGTAT
ARR2-R			GCCTATTGCGCATAAAATGG
OXA-10F	bla_{OXA-10}	261	TTAGGCCTCGCCGAAGCG
OXA-10R			CTTTGTTTTAGCCACCACCAATGATG

F, forward; R, reverse primer

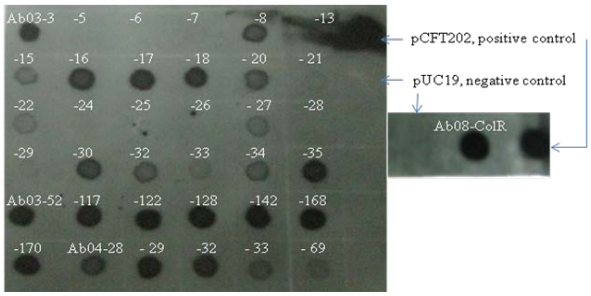

Fig 1: Dot blot hybridization using the *intI1* probe for the detection of *intI1* gene. Thirty isolates were positive for *intI1* gene. pCFT202 carrying class 1 integron element was used as a positive control and plasmid pUC19 was used as negative control.

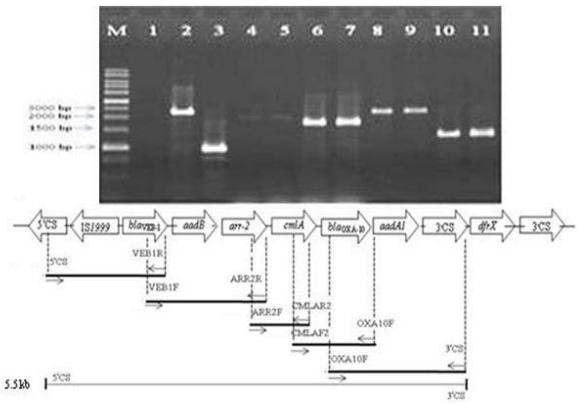

Figure 2: PCR mapping of bla_{VEB-1} containing class 1 integron element from isolate Ab03-168. Lane M=Molecular weight marker (1Kb+, invitrogen), Lane 1= Negative control, Lanes 2, 4, 6, 8, and 10 are positive control for fragments 5'CS-bla_{VEB-1}, bla_{VEB-1}-*arr-2* , *arr-2-cmlA*, *cmlA*-bla_{OXA-10} , and bla_{OXA-10} -3'CS from Lane 3= 5'CS- bla_{VEB-1} from *P. aeruginosa* strain 58. Lanes 3, 5, 9, and 11 represents fragments bla_{VEB-1}-*arr-2*, *arr-2-cmlA*, *cmlA*-bla_{OXA-10}, and bla_{OXA-10} -3'CS from isolate Ab03-168).

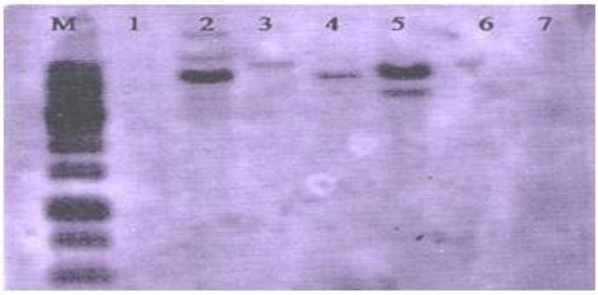

Figure 3 Genetic localization of bla_{VEB-1} gene in bla_{VEB-1} gene positive isolates. Signals were detected only in four lanes. The fragments generated using *Eco*RI was different in different isolates suggesting different location of bla_{VEB-1}. Lane M= Molecular weight marker (1 kb+, SibZymes), Lane 2-5 represents isolates; Ab03-52, Ab03-117, Ab03-122, and Ab03-168, respectively.

Moreover, two isolates (Ab08-25 and -26) were also *intI1*positive by *intI1* PCR. Altogether, 32 isolates were positive for class 1 integron element (86.48%). PCR amplification detected bla_{VEB-1}, bla_{OXA-10}, *arr-2*, and *cmlA* in 7 (Ab08-15, Ab03-52, -117, -122, -168, Ab04-29, Ab04-32), 10 (Ab08-20, -25, -26, -27, -29, Ab03-52, -117, -122, -168, Ab04-29), 5 (Ab08-15, Ab03-117, -168, Ab04-29, -32), and 9 (Ab08-15, -16, -21, Ab03-52, -117, -122, -168, Ab04-29, -32) isolates, respectively from *intI1* positive isolates.

Amplification of variable region of class 1 integron element

The variable region of class 1 integron element was amplified from 32 *intI1* positive isolates using the consensus primer set 5'CS and 3'CS. Numerous variable regions ranging from 0 to 5 were amplified (data not shown). Smallest variable region amplified was 0.5 kb (n=16) and the largest was 5.5 kb (n=4).

Commonest variable region that was amplified was 0.7 kb (n=23). Variable region sized 5.5 Kb was amplified from 4 isolates (Ab03-168, Ab04-28, Ab08-20, and Ab08-22) and only isolate Ab03-168 was positive for *bla*$_{VEB-1}$.

Characterization of *bla*$_{VEB-1}$ containing integron element

The only class 1 integron element carrying the *bla*$_{VEB-1}$ gene with a variable region of 5.5 Kb in an isolate Ab03-168 was characterized by PCR mapping (5'CS-VEB1R, VEB1F-ARR 2R, ARR 2F-CMLA R2, CMLA F2-OXA-10R, and OXA-10R-3'CS). The gel electrophoresis of amplicons detected is shown in Fig 2. The class 1 integron element revealed was 5'CS-*aadB-bla*$_{VEB-1}$- *arr-2-cmlA-aadA1-bla*$_{OXA-10}$-3'CS.

bla$_{VEB-1}$ localization

Southern blot was performed with *bla*$_{VEB-1}$ specific probe to interrogate the genetic localization of *bla*$_{VEB-1}$ in *bla*$_{VEB-1}$ positive isolates (n=7). The signal was detected for 4 isolates (Fig 3). The signals generated were different in different isolates, two to three bands (Ab03-52, Ab03-168) and a single band (Ab03-117, Ab03-122). The genetic environment surrounding either at upstream or downstream of *bla*$_{VEB-1}$ gene was different in different isolates.

DISCUSSION

The class 1 Integron element harbors multiple resistance cassettes and confer resistance to numerous antibiotics. This is a hallmark of resistance phenotype in *A. baumannii*.[1] The existence of this mobile genetic element was sought by dot blot hybridization and *intI1* PCR. The prevalence of class 1 integron element in our isolates was 86.48%. High prevalence of class 1 integron element have also been reported elsewhere.[12,13] Multidrug resistance pattern of these isolates might have correlated with the presence of this integron element.[11]

The largest class 1 integron element in GRI, AbaR1 is 5.5 kb which is present in European clone I, AYE and harbors multiple resistant cassettes (*bla*$_{VEB-1}$, aadB, *arr-2*, *cmlA bla*$_{OXA-10}$, and *aadA1*).[1] The resistant cassettes (*bla*$_{VEB-1}$, *arr-2*, *cmlA and bla*$_{OXA-10}$) and variable regions of class 1 integron element from these isolates were amplified. The resistant cassettes; *bla*$_{VEB-1}$ (n=7), *bla*$_{OXA-10}$ (n=9), *arr-2* (n=10), and *cmlA* (n=5) were detected. All of these isolates were also positive for *intI1*. The variable region of 5.5 kb was amplified in 4 isolates (Ab08-20, -22, Ab04-168, and Ab04-28) while *bla*$_{VEB-1}$ gene was only present in an isolate, Ab08-168. The integron from this isolate was characterized by PCR mapping. The cassette found was 5'CS-*intI1-bla*$_{VEB-1}$-*aadB-arr-2-aadA1-cmlA-bla*$_{OXA-10}$-3'CS. Gene cassettes, *aadB* and *aadA1* were not amplified in this study however, *bla*$_{VEB-1}$-*arr-2* (2000 bp) and *bla*$_{OXA-10}$-3'CS (1500 bp) amplicons were of accurate size to accommodate for *aadB* and *aadA1*, respectively.

The common structure of class 1 integron element that is widely disseminated among *Enterobacteriaceae* is 5'CS-IS*1999-bla*$_{VEB-1}$-*aadB-arr-2-bla*$_{OXA-10}$-*aadA1-cmlA5*-3'CS but variations have been reported.[1] The cassette organization that was found in Ab03-168 was similar to European clone I, AYE, except IS*1999* was missing between 5'CS and *bla*$_{VEB-1}$ (Figure 2). Tribuddharat *et al*. described IS*1999* element primarily in *P. aeruginosa* from Thailand and was integrated upstream of *bla*$_{VEB-1}$ gene cassette.[9] This insertion sequence provides a strong promoter for the expression of this gene.[14] Since, isolate Ab03-168 possess carbapenemase OXA-23 and can hydrolyze extended spectrum cephalosporins (ESCs), IS*1999* might not be required for high level expression of *bla*$_{VEB-1}$.[1] Furthermore, carbapenems are the first choice of treatment for this superbug so the withdrawal of ESCs might have helped IS*1999* to shuffle around the genome. The integron structure without IS*1999* was seen in *P. aeruginosa* from France.[15] These variations were further supported by our finding that other isolates; Ab08-15, Ab03-52, -117, -122, Ab04-29, and -32 were also positive for *bla*$_{VEB-1}$ but, neither of these isolates was positive for integron element of size 5.5 kb. The *bla*$_{VEB-1}$ was localized by southern blot in isolates carrying *bla*$_{VEB-1}$ and the genetic environment of *bla*$_{VEB-1}$ was different in different isolate. This also suggests that the genetic make up of isolates carrying the *bla*$_{VEB-1}$ gene was different and hence different integron structure.

The integron element with slight variation was widely disseminated in other Gram-negative bacteria like; *P. aeruginosa, E. coli, E. cloacae, and K. pneumoniae* in this hospital and abroad.[9,10] This suggests the interspecies horizontal gene transfer and international dissemination of this mobile genetic element among international clones of *Enterobacteriaceae* and minor variation brought about by selective antibiotic pressure in different geographical areas.[16]

CONCLUSIONS

bla$_{VEB-1}$ containing class 1 integron element with

cassette organization similar to the one present in *A. baumannii*, European clone I, AYE was identified in Thai *A. baumannii*. Presence of common integron element in international clones of *A. baumannii* isolates implies the mating of our clones with international clones at least once or spread of our clones internationally. Monitoring and surveillance of the global spread of multi-resistant *A. baumannii* is mandatory to control the spread of resistant gene cassettes and this multi-resistant pathogen.

CONFLICT OF INTEREST: None to declare

FINANCIAL INTEREST: None to declare

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