

HIGH PREVALENCE OF bla_{OXA-23} IN OLIGOCLONAL CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII* FROM SIRIRAJ HOSPITAL, MAHIDOL UNIVERSITY, BANGKOK, THAILAND

Badri Thapa, Chanwit Tribuddharat, Somporn Srifuengfung
and Chertsak Dhiraputra

Department of Microbiology, Faculty of Medicine Siriraj Hospital,
Mahidol University, Bangkok, Thailand

Abstract. *Acinetobacter baumannii* has emerged in health care settings as a pandrug-resistant pathogen. Carbapenems are ineffective for treatment of this pathogen. Here we explored the molecular epidemiology and mechanism of carbapenem resistance in clinical isolates of carbapenem-resistant *A. baumannii* (CRAB). Antibiotic susceptibility by disk diffusion test was performed using imipenem and meropenem disk on 200 different clinical CRAB isolates. All isolates were resistant and gave inhibition zones of both antibiotic disks ≤ 13 mm. Polymerase chain reaction (PCR) was carried out on 37 randomly selected isolates to amplify the common carbapenem hydrolyzing β -lactamase genes (bla_{OXA-23} -like, $bla_{OXA-24/40^-}$ -like, bla_{OXA-58} , bla_{IMP} , and bla_{VIM}). Clones were resolved by PCR-randomly amplified polymorphic DNA (PCR-RAPD) and plasmid profiling. PCR amplification and DNA sequencing revealed the existence of bla_{OXA-23} downstream of the insertion element, ISAb_a1, in all 37 isolates tested. This segment was present in the carbapenem-resistant genomic island AbaR4. These isolates were resolved into three RAPD types (Type I, 20 isolates; Type II, 16 isolates; and type III, 1 isolate) and 10 plasmid profiles. The CRAB isolates investigated here were oligoclonal and carbapenem resistance was conferred by the presence of bla_{OXA-23} . The presence of this β -lactamase gene in many clonal isolates indicated its wide spread.

Key words: *A. baumannii*, β -lactamase gene, carbapenem resistance, CRAB

INTRODUCTION

Acinetobacter baumannii is an emerging nosocomial pathogen as it can survive very well in various salinity, pH and humidity environments. Furthermore, some

Correspondence: Chanwit Tribuddharat, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Prannok Road, Bangkok 10700, Thailand.

Tel 66 (0) 2419 9686; Fax : 66 (0) 2411 3106

E-mail: sictb@mahidol.ac.th

isolates have become pandrug-resistant and persisted in hospital environment (Bergogne-Berezin and Towner, 1996). *A. baumannii* is responsible for 3-5% of nosocomial pneumonia, one of the most common presentations in mechanically ventilated patients in intensive care units (ICU) (CDC, 1987; Dijkshoorn *et al*, 1993). Wound infections, bacteremia, urinary tract infection, secondary meningitis, infective valve endocarditis, peritonitis, osteomyelitis, and keratitis also have been reported

(Bergogne-Berezin and Towner, 1996). A mortality rate of 30-75% has been reported for *Acinetobacter* spp pneumonia in hospitalized patients and even more in ventilator assisted patients (Scott *et al*, 2007). In 2002, the prevalence rate of this pathogen in Siriraj Hospital, Mahidol University, Bangkok, Thailand was 8% with a mortality rate of 50% (Keerasuntonpong *et al*, 2003).

Carbapenems are the drugs of choice for this pathogen and carbapenem-resistant *A. baumannii* (CRAB) has been frequently encountered (Barbolla *et al*, 2003; Dalla-Costa *et al*, 2003; Manuel, 2003). The National Antimicrobial Resistance Surveillance Center, Thailand (NARST) reported that susceptibility to imipenem decreases from 95% to 65%, and a study from 42 hospitals across the country showed that *A. baumannii* is the third commonest nosocomial pathogen among all gram-negative bacteria (Surasarang *et al*, 2007). Among these, CRAB isolates are rising (Jamulitrat *et al*, 2007). One of the major mechanisms of carbapenem resistance in this pathogen is the production of carbapenem hydrolyzing β -lactamases. These specific groups of β -lactamases are categorized into class B metallo β -lactamases (MBLs) (IMP, VIM), and class D (oxacillinases) (OXA-23-like, OXA-24/40-like, and OXA-58) (Ambler *et al*, 1991; Naas and Nordmann, 1999). Other mechanisms include efflux pump, loss or low level expression of 29 kDa (CarO), 33-39 kDa, and 43kDa (homologous to *P. aeruginosa* imipenem resistance protein, OprD) proteins, and altered affinity in penicillin binding proteins (PBPs) (Vila *et al*, 2007). Several outbreaks, especially in intensive care units, have been reported city-, country-, and continent-wise in recent years (Barbolla *et al*, 2003; Dalla-Costa *et al*, 2003; Manuel *et al*, 2003). Isolates of *A. baumannii* producing

oxacillinases, OXA-23, OXA-24 and OXA-58, are prevalent in Europe and Americas (Bou *et al*, 2000; Heritier *et al*, 2005; Villegas *et al*, 2007), while OXA-23-producing strains are highly prevalent in Asia-Pacific region including Thailand (Mendes *et al*, 2009). MBL, eg, *bla*_{IMP} variants, producing strains are widespread in all continents, but *bla*_{VIM-2} is restricted to South Korea (Yum *et al*, 2002; Poirel and Nordmann, 2006).

Here we investigated the molecular epidemiology and mechanism of carbapenem resistance in CRAB, contributed mainly by a carbapenem hydrolyzing OXA β -lactamase in Thai endemic oligoclonal isolates of *A. baumannii*.

MATERIALS AND METHODS

Bacterial strains and antibiotic susceptibility test (AST)

Two hundred different clinical isolates of CRAB from years 2003, 2004, 2006, and 2008 were isolated in Siriraj Hospital, Bangkok, Thailand by monitoring carbapenem susceptibility using the disk diffusion test (DDT) on Mueller-Hinton agar plates with imipenem (10 μ g) and meropenem (10 μ g) disks based on Clinical Laboratory Standards Institute guidelines (CLSI, 2005). Disk diffusion tests were performed with the following 12 antibiotics (μ g/disk): trimethoprim/sulfamethoxazole (1.25/23.75), ciprofloxacin (5), gentamicin (30), amikacin (30), netilmicin (30), cefotaxime (30), ceftriaxone (30), ceftazidime (30), cefepime (30), piperacillin-tazobactam (100+10), tigecycline (10), and colistin (10). Manufacturer's guidelines were followed to interpret the zone of inhibition based on CLSI guidelines. These CRAB isolates were from different clinical wards and from various specimen sources, eg, blood, sputum, urine, and various catheters. Thirty seven isolates, which included 7 from the year

2003, 5 from 2004, 1 from 2006, and 24 from 2008 were chosen randomly. All isolates were identified by biochemical tests according to Bouvet and Grimont (1987). These isolates were further confirmed by amplification of naturally occurring *bla*_{OXA-51}-like gene as described below (Ruiz *et al.*, 2007). *Pseudomonas aeruginosa* ATCC 27853 was used as a control strain for the disk diffusion test.

DNA extraction, polymerase chain reaction (PCR) and DNA sequencing

Genomic and plasmid DNA were extracted using commercial DNA extraction kits (Puregene, Minneapolis, MN, USA; Nucleospin Plasmid, Germany) following the manufacturers' guidelines, and DNAs were stored in -20°C until used. PCR reactions were performed for the detection of carbapenem β-lactamase genes with sequence specific primers (Table 1). PCR amplification was performed in 50 µl reaction volume containing 0.2 µM of each primer, 2 mM 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 conducted in Perkin-Elmer thermocycler 2400 model using the following thermocycling profile: initial heating at 95°C for 5 minutes; 30 cycles of 95°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute/1,000 nucleotides; and a final heating at 72°C for 7 minutes. Amplified products were detected by agarose gel-electrophoresis in 1% Tris-acetate-EDTA (TAE) agarose (Research Organics, VA) and staining with ethidium bromide.

A. baumannii, Ab08-ColR, was subjected to whole genome pyrosequencing (GS-FLX, Roche). Contigs were assembled using Roche 454 sequence assembly tool. Obtained contigs were analyzed using the tools at <http://www.ncbi.nlm.nih.gov> and

sequence data used here were obtained from this genome sequence. The nucleotide sequences of the ISAbA1-*bla*_{OXA-23} fragment have been deposited as GenBank accession nos. FJ959346 and GQ268326.

Clone analysis

All isolates were resolved at clonal level by PCR-randomly amplified polymorphic DNA (PCR-RAPD) and plasmid profile analysis. PCR-RAPD protocol was as previously described (Hansen *et al.*, 1998) with slight modification. In brief, the reaction volume of 20 µl contained 50 ng of genomic DNA template, 0.2 µM primer (R003, 5' CCTTGACGCA 3'), 0.2 mM dNTPs (FINZYMES), 2.5 µl of supplied PCR buffer, and 1.0 U of *Taq* polymerase (FINZYMES). Dimethylsulfoxide was added into the reaction at the final concentration of 5%. The thermocycling profile was as follows: initial heating at 94°C for 2 minutes; followed by 40 cycles of 94°C for 10 seconds, 36°C for 30 seconds, and 72°C for 1 minute; and a final heating at 72°C for 2 minutes. Amplified products and extracted plasmids were resolved in 1% TAE agarose. Plasmid profiles were interpreted on the basis of the number and size of the plasmids.

RESULTS

Carbapenem susceptibility test

CLSI criterion for carbapenem susceptibility for *A. baumannii* is an inhibition zone size ≥16 mm. Inhibition zones for *A. baumannii* in this study ranged from no zone to 11 mm, with the majority (56.7%) having a zone of inhibition ≤9 mm.

Resistant phenotypes of CRABs

Based on 14 antibiotics tested, 37 isolates could be typed into 7 resistant phenotypes (Table 2). All CRABs were resistant to all β-lactams and ciprofloxacin. They also

Table 1
Primers for amplification of carbapenemase genes^a.

Primer name	Target gene	Sequence, 5'-3'	Amplicon (bp)	GenBank acc no.	Nucleotide position
OXA-23F	ISAbA1- <i>bla</i> _{OXA-23}	GATGTGTCATAGTATTCTCG	1,065	CP001182	585729-49
OXA-23R		TCACAAACAACAAAAGCACTG			586793-73
OXA-24F	<i>bla</i> _{OXA-24/40} like	TTCCCCTAACATGAATTGT	1,023	AY228470	1245-64
OXA-24R		GTACTAATCAAAGTTGTGAA			2247-66
OXA-58F	<i>bla</i> _{OXA-58}	ACAGCTTATATTCCCTGCATCTACATT	257	CP000864	13612-37
OXA-58R		GCCTATTGCATATTGCCATAAC			13869-47
OXA51U	<i>bla</i> _{OXA-51,-69, -71, 75, -78}	AACAAGCGCTATTTATTCAG	641		
OXA51L		CCCATCCCCAACCAACCACTT			
ISAbA1F	ISAbA1- <i>bla</i> _{OXA-23}	ATGATGAGCGCAAAGCACT	228	CP001182	585653-71
OXA23R2		TGCTGAACCGTACAACCAGA			585908-887
IMPF	<i>bla</i> _{IMP}	TTGCCAGATTAAAAAT	570	EF375699	64-80
IMP003		ACCAAGTTTGCCTTACCAT			613-32
VIMFW	<i>bla</i> _{VIM}	GTCTACCCGTCCAATGGTCTCA	550	EU419746	196-17
VIMRV		AGCAAGTCTAGACCGCCCG			728-46

^aPrimers were designed in this study except for OXA51 primers which was described by Ruiz *et al*, 2007.

showed 100% resistance to piperacillin-tazobactam and trimethoprim-sulfamethoxazole. Although most isolates were susceptible to colistin and tigecycline, a few numbers of isolates were resistant to tigecycline (Ab08-32 and Ab03-33) and colistin (Ab08-17, -29, -ColR). Resistance to aminoglycosides was variable.

PCR detection and DNA sequencing of carbapenemase gene

PCR was carried out for detection of carbapenem β-lactamase genes. Gene specific primers were designed for carbapenem β-lactamase genes that are prevalent in many regions, viz. *bla*_{OXA-23}, *bla*_{OXA-24/40}, *bla*_{VIM} and *bla*_{IMP} (Mendes *et al*, 2009). Since class D β-lactamase gene, *bla*_{OXA-23}, is flanked upstream by the ISAbA1 insertion element (Turton *et al*, 2006), the primers were designed to amplify from the frag-

ment of ISAbA1 to obtain a complete open reading frame of *bla*_{OXA-23} gene (Fig 1). All 37 isolates contained *bla*_{OXA-23}, flanked upstream by ISAbA1. The IRL indicates left indirect repeat (GenBank acc. no. EU827526). The nucleotide sequence data obtained from pyrosequencing of isolate Ab08-ColR confirmed the gene orientation of ISAbA1-*bla*_{OXA-23}. This segment was found in the carbapenem-resistant genomic resistant island, AbAR4, (Fig 2) which has been identified in *A. baumannii* strain, AB0057, from Walter Reed Army Medical Center, USA (Adams *et al*, 2008) and strain U437 from Ireland (GenBank acc no. EU827526).

Clone analysis

Clonal relationship was investigated using PCR-RAPD, which resolved the CRABs into three RAPD types (I-III) (Fig 3).

Table 2
PCR-RAPD, plasmid profile, and resistance phenotype of CRABs studied.

Plasmid profile	I						II						III			
	A	B	C	D	E	F	G	H	J	untyp	A	B	D	F	I	E
Resistant profile	i	ii	i	ii	iii	i	iii	i	v	vii	ii	i	vi	i	iv	i
Isolate	Ab08	Ab08	Ab03	Ab08	Ab08	Ab03	Ab04	Ab03	Ab08	Ab04	Ab04	Ab03	Ab08	Ab08	Ab04	Ab08
	-06,-08,-03,-17,-22,-21	-52,-29,-32,-122	-33,-32,-33	-32,-33	-128	-33	-168	-170	-117	-142	-69	-29	-07,-15,-30	-28	-20	-32,-13

Resistant phenotypes: i, sensitive to TG, CT, NET; ii, sensitive to TG, CT; iii, sensitive to CT, iv, sensitive to TG, CT, AK, NET; v, sensitive to TG; vi, sensitive to TG, AK, NET; vii, sensitive to TG, NET. Abbreviations: TG, tigecycline; CT, colistin; NET, netilmicin; AK, amikacin. CN, gentamicin; IMP, imipenem; MEM, meropenem. Untyp, untypeable.

Twenty of the isolates (45%) were typed as RAPD type I, 16 isolates (43%) type II, and only one isolate, Ab08-13, represented type III (Table 2). RAPD type I consisted of isolates obtained in 2003, 2004, and 2008. All isolates isolated in the year 2003 were of type I. Type II represented isolates from 2004 and 2008, and an isolate from 2006 (Ab06-35).

Plasmids varied in numbers and sizes, which could be divided into ten profiles (A to J) (Fig 4, Table 2). Nineteen RAPD type I isolates were grouped into 7 plasmid profiles (type A, 6 isolates; type B, 5 isolates; type C, 2 isolates; type D, 3 isolates (including Ab08-ColR); type G, 1 isolate; type H, 1 isolate; type J, 1 isolate). The 16 RAPD type II isolates were grouped into 5 plasmid profiles, mainly ($n=11$) plasmid profile A (Table 2). The single isolate with RAPD type III (Ab08-13) had plasmid profile E. The plasmid profile for Ab04-29 (RAPD type I) was not obtained in spite of many attempts.

DISCUSSION

Pandrug-resistant *A. baumannii* has been isolated frequently in nosocomial outbreaks especially in intensive care units throughout the world including Thailand (Chaiwarith *et al*, 2005). Its abilities to acquire a broad range of resistance genes and to survive in various ecological niches are the reasons

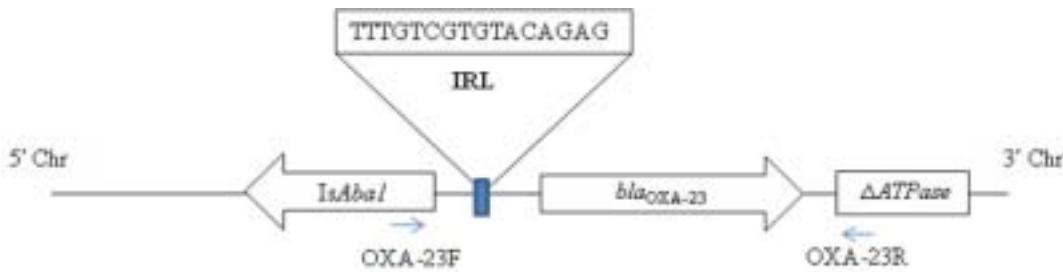


Fig 1—Genetic location of *bla*_{OXA-23}. *Bla*_{OXA-23} is flanked upstream by *ISAbal*. IRL indicates left indirect repeat; Right direct repeat, right indirect repeat, and left direct repeat are not shown.

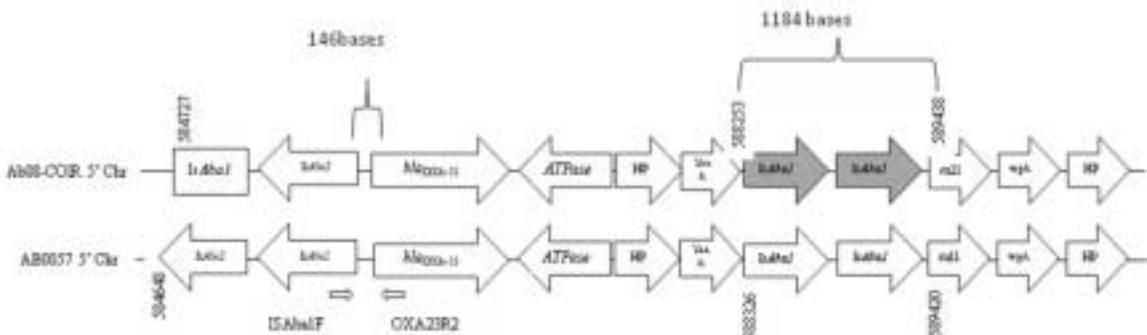


Fig 2—Genetic map of genomic resistance island AbaR4 identified in Ab08-ColR using pyrosequencing. AbaR4 in Ab08-ColR has been compared with AbaR4 identified in *A. baumannii* strain, AB0057 (European clone I) (GenBank acc. no CP001182). The open reading frames and the orientation of genes are shown. ORFs shaded with gray (region 588254-589437) and 146 bases between *ISAbal* and *bla*_{OXA-23} were not obtained from contig assembly using pyrosequencing data. The gap between *ISAbal* and *bla*_{OXA-23} was filled using the primer pairs *ISAbalF* and *OXA23R2*. The box at 5' region of AbaR4 in Ab08-colR represents partial sequence of *ISAbal* element.

for this pathogen's ubiquity in such an environment. Investigation of skin flora of patients at Siriraj Hospital revealed mainly *A. baumannii* and *Staphylococcus aureus* (Visanu *et al.*, 2003). In 2002, multidrug-resistant (including carbapenem resistant) *A. baumannii* isolated from infected hospitalized patients was 52% (Tribuddharat *et al.*, 2003) and incidence of CRABs is still rising. Carriage of these pathogens in the skin and also the ability to isolate this patho-

gen from hospitalized patients mean that they are ready to become a predominant organism, when patients are treated with antibiotics. Once the organism becomes established on severely ill patients' bodies, any invasive procedures would have deadly outcome due to their compromised immunity. The CRAB isolates studied here were multidrug resistant. Tigecycline and colistin still remain the choice of treatment for this pathogen.

MOLECULAR STUDY OF CARBAPENEM RESISTANCE IN CRAB

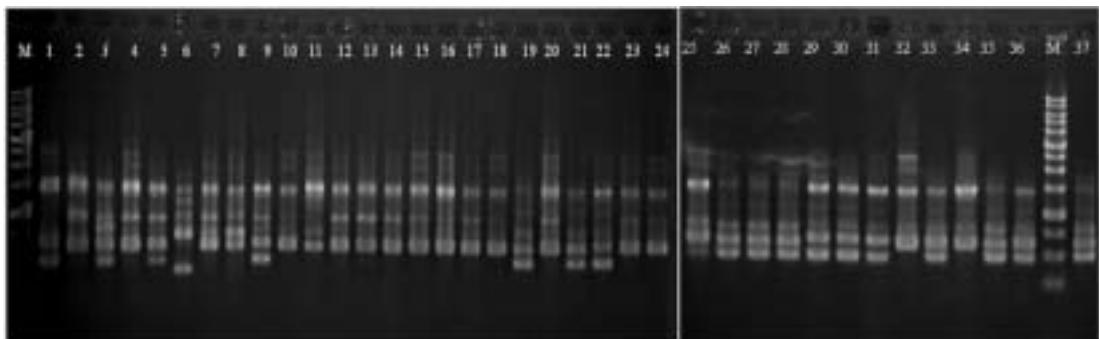


Fig 3-PCR-RAPD types of 37 CRAB isolates. M is molecular weight markers (1 kb⁺, Invitrogen). Lanes 1-37 are Ab08-03, Ab08-05, Ab08-06, Ab08-07, Ab08-08, Ab08-13, Ab08-15, Ab08-16, Ab08-17, Ab08-19, Ab08-20, Ab08-21, Ab08-22, Ab08-24, Ab08-25, Ab08-26, Ab08-27, Ab08-28, Ab08-29, Ab08-30, Ab08-32, Ab08-33, Ab08-34, Ab06-35, Ab08-ColR, Ab03-52, Ab03-117, Ab03-122, Ab03-128, Ab03-142, Ab03-170, Ab04-28, Ab04-29, Ab04-32, Ab04-33, Ab04-69, and Ab03-168, respectively. Ab03-, Ab04-, Ab06-, and Ab08- indicate the year of isolate collection (2003, 2004, 2006, and 2008, respectively). The isolates were typed into 3 RAPD types. Type I corresponds to lanes 1, 3, 5, 9, 12, 13, 19, 21, 22, 25-31, 33, 35-37; type II to lanes 2, 4, 7, 8, 10, 11, 14-18, 20, 23, 24, 32, and 34. Type III is shown in lane 6.



Fig 4-Plasmid profiles of 36 CRAB isolates. M is molecular weight marker (1 kb⁺, Invitrogen). Lanes 1-39 are Ab08-03, Ab08-05, Ab08-06, Ab08-07, Ab08-08, Ab08-13, Ab08-15, Ab08-16, Ab08-17, Ab08-19, Ab08-20, Ab08-21, Ab08-22, Ab08-24, Ab08-25, Ab08-26, Ab08-27, Ab08-28, Ab08-29, Ab08-30, Ab08-32, Ab08-34, Ab08-35, Ab08-33, Ab03-52, Ab03-117, Ab03-122, Ab03-128, Ab03-142, Ab03-168, Ab03-170, Ab04-28, Ab04-29, Ab04-32, Ab04-33, and Ab04-69, respectively. The year of isolate collection is as described in legend for Fig 3. Isolates were typed into 10 plasmid profiles. Type A corresponds to the lanes 1-5, 7, 8, 9, 10, 12-15, 17, 20, 22, 23, 32; type B to lanes 18, 19, 21, 24, 25, and 27; type C to lanes 28 and 35; and type D to lanes 11, 30, and 31. Lane 6, 16, 26, 29, 34, and 36 represents for type E, F, G, H, I and J, respectively. Ab04-29 (lane 33) was untypeable. Ab08-ColR also has plasmid profile type D (data not shown).

The mechanisms underlying the carbapenem resistance are either from spontaneous change (low level expression of drug-influx porins, alteration of penicillin binding proteins, or increased efflux

pumps) or horizontally acquired (ability to produce carbapenem hydrolyzing enzymes). Here, we investigated the mechanism for carbapenem resistance and showed that resistance was due to *bla*_{OXA-23}

gene. This gene was initially named ARI-1 and is the first carbapenem hydrolyzing oxacillinase to be reported in *A. baumannii* (Panton *et al*, 1993). All *bla*_{OXA-23} identified was flanked upstream by the ISAb1 insertion element, which provides a strong promoter region for the high level expression of this gene (Turton *et al*, 2006). This fragment is located in the genomic resistant island AbaR4 (Adams *et al*, 2008). In this study, pyrosequencing data from Ab08-ColR also showed the presence of this carbapenem resistant island.

Although the PCR detection method did not indicate the status of oxacillinase expression level, the inhibition zone sizes, which ranged form no zone to a narrow zone, implied high levels of expression. Other above mentioned intrinsic mechanisms might act synergistically (Vila *et al*, 2007). High prevalence of CRAB carrying only *bla*_{OXA-23} genes suggests the better fitness of this pathogen to survive this particular hospital environment. The primer pairs OXA-23F and OXA51L were used to amplify the ISAb1-*bla*_{OXA-51}-like gene and the amplification was negative for all isolates. Thus the endogenous *bla*_{OXA-51}-like gene does not have any role in carbapenem resistance in these isolates. No isolates in this study were found to carry other oxacillinases (*bla*_{OXA-24/40}-like, *bla*_{OXA-58}) or MBLs. However, more clinical isolates are needed to be tested to confirm the true absence of MBLs and other carbapenem hydrolyzing oxacillinases.

Genetic arrangement of *bla*_{OXA-23}, flanked upstream by ISAb1 showed that this segment is most likely mobile and highlights its acquisition by horizontal gene transfer. Further study on the transposition of this mobile genomic resistance island and *bla*_{OXA-23} gene through horizontal gene transfer would be worthwhile. Furthermore, this class D carbapenem β -

lactamase gene has been identified as plasmid borne and this may have been the reason for its broad dissemination (Poirel and Nordmann, 2006). Common use of antibiotics in the clinical setting creates antibiotic selective pressure that favors the transfer of resistant plasmids (Davies, 1994). Chromosomal integration of the resistance determinants might have occurred as shown in databases from genome analyses of many *A. baumannii*. Thus, the spread of these resistance determinants might also be by vertical gene transfer.

In this study, we report 100% prevalence rate of *bla*_{OXA-23} in CRAB, and this may reflect the collection of repetitive clones. Similar high prevalence of CRAB carrying *bla*_{OXA-23} has been reported elsewhere and in Thailand (Dalla-Costa *et al*, 2003; Jeon *et al*, 2005; Naas *et al*, 2005; Mendes *et al*, 2009, Nuimsup *et al*, 2009). All isolates collected in this study were isolated from different patients in different wards and from various specimens, so they were from multiple sources. RAPD and plasmid profiles suggested they were oligoclonal. The compositions of drug resistance determinants were different and unique in each isolate as investigated by PCR (data not shown). This also suggests dynamic genetic changes of these isolates, although other low discriminatory typing assays, if were they used, such as multilocus sequence typing or ribotyping, may also show their high relatedness.

PCR-RAPD is an easy, fast and cost effective molecular typing tool, which can be used by all technical staff and laboratories. Interpretation of the data is easy and does not need special expertise. This technique can be a tool for clonal analysis in outbreaks, where more sophisticated tools are not available and epidemiological answers are needed in a short span of time. The isolates collected in the years 2003,

2004, 2006, and 2008 were analyzed by PCR-RAPD and typed into 3 PCR-RAPD types. Type I and type II were present in 2003 and also in 2008, which reflects the endemicity of these clones the spread of these successful clones over the years. In the year 2003 and 2004, only these 2 RAPD types were circulating, but identification of RAPD type III (Ab08-13) in 2008 may indicate a new introduction. This hospital is a tertiary care center, and both inter- and intra-hospital transfer of patients frequently occurs and the oligoclonal nature of isolates reflects this situation. Similar reports have been described regarding the polyclonal outbreak of CRAB (Jeon *et al.*, 2005; Naas *et al.*, 2005).

Plasmids may be markers of drug resistant phenotypes, especially in multi-drug-resistant pathogens isolated from hospitals. We demonstrated the presence of numerous plasmids, which enabled us to place the 3 RAPD types into 10 plasmid profiles. Most isolates with plasmid profile A ($n = 12$) belonged to RAPD type II, while fewer isolates ($n = 6$) represented RAPD type I. Isolates with the same plasmid profile but different RAPD types might reflect genetic exchanges among these isolates. Based on the resistant patterns, isolates with unique plasmid profiles could be typed into different resistant phenotypes (Table 2). Although plasmid profiling was more discriminatory than RAPD, a drawback of using plasmid profiles as a molecular typing tool is that they are highly dynamic and may be lost in some isolates, as in Ab08-20, Ab03-168, Ab03 -170, and Ab08-ColR. However, in this study, most *A. baumannii* isolates harbored numerous plasmids, and they can still be used to type the multidrug-resistant pathogens.

In summary, the mechanism of resistance to carbapenem in all CRAB isolates

was the presence of chromosomal *bla*_{OXA-23} located in the genomic resistant island AbaR4, and this gene was widespread in oligoclonal isolates of *A. baumannii*. Location of this gene in a carbapenem resistant island, flanked upstream by ISAb1, strongly suggests acquisition by a horizontal transfer, and was likely to be widely disseminated further through vertical transfer. Strict monitoring and surveillance of CRAB is necessary to prevent spread of this pathogen. Further revision of treatment guidelines, proper education of the health care givers, and stringent infection control strategies are the key points to be addressed in order to overcome the burden derived from this pathogen. Future studies should also aim at developing novel drugs, and in the present scenario where global CRAB prevalence is rising, specific carbapenemase inhibitors seem to be likely candidates.

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