Original Article

ตรวจสอบเอนไซม์คาร์บาพีนีเมสและยีนกลุ่ม *bla*$_{OXA-23}$ ในแบคทีเรีย *Acinetobacter baumannii* ที่ดื้อต่อยากลุ่มคาร์บาพีเนมในโรงพยาบาลสรรพสิทธิประสงค์

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บทคัดย่อ

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บทนำ: *Acinetobacter baumannii* คือเชื้อแบคทีเรียแกรมลบที่เป็นสาเหตุของการติดเชื้อในโรงพยาบาลที่มีอัตราการดื้อต่อยาปฏิชีวนะสูง มีรายงานการเพิ่มขึ้นของความชุกของแบคทีเรีย *A. baumannii* ที่ดื้อต่อยากลุ่มคาร์บาพีเนมทั่วโลก วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อศึกษาอัตราการดื้อยาต้านจุลชีพของแบคทีเรีย *A. baumannii* ที่แยกได้จากสิ่งส่งตรวจทางคลินิกของผู้ป่วยในของโรงพยาบาลสรรพสิทธิประสงค์ จังหวัดอุบลราชธานี และตรวจสอบการสร้างเอนไซม์คาร์บาพีนีเมสกลุ่ม OXA-23 ของแบคทีเรีย *A. baumannii* ที่ดื้อยากลุ่มคาร์บาพีเนม **วัสดุและวิธีการทดลอง:** ทดสอบความไวต่อยาต้านจุลชีพของแบคทีเรีย *A. baumannii* จำนวน 33 ไอโซเลต ด้วยวิธี agar disk diffusion คัดเลือกแบคทีเรีย *A. baumannii* ที่ดื้อยากลุ่มคาร์บาพีเนม จำนวน 14 ไอโซเลต เพื่อนำไปตรวจสอบการสร้างเอนไซม์คาร์บาพีนีเมสด้วยวิธี modified Hodge test ตรวจสอบยีนกลุ่ม *bla*$_{OXA-23}$ ในแบคทีเรีย *A. baumannii* ดื้อยากลุ่มคาร์บาพีเนมด้วยปฏิกิริยาลูกโซ่พอลิเมอเรส นำชิ้นส่วนดีเอ็นเอที่ได้ไปทำให้บริสุทธิ์ และหาลำดับนิวคลีโอไทด์ **ผลการทดลอง:** การทดสอบความไวของต่อยาต้านจุลชีพของแบคทีเรีย *A. baumannii* จำนวน 33 ไอโซเลต พบแบคทีเรีย *A. baumannii* ที่ดื้อยากลุ่มคาร์บาพีเนม จำนวน 16 ไอโซเลต คิดเป็นร้อยละ 48.5 อัตราการดื้อต่อยา imipenem และ meropenem เท่ากับร้อยละ 45.2 และ ร้อยละ 61.5 ตามลำดับ ร้อยละของแบคทีเรียที่สร้างเอนไซม์คาร์บาพีนีเมสเท่ากับร้อยละ 71.4 (10 จาก 14) จากการตรวจสอบยีนกลุ่ม *bla*$_{OXA-23}$ ในแบคทีเรีย *A. baumannii* ดื้อยากลุ่มคาร์บาพีเนม จำนวน 14 ไอโซเลต ตรวจพบยีนกลุ่ม *bla*$_{OXA-23}$ ในแบคทีเรียส่วนใหญ่ คิดเป็นร้อยละ 85.7 **สรุปผล:** การศึกษาครั้งนี้รายงานข้อมูลอัตราการดื้อยาต้านจุลชีพของแบคทีเรีย *A. baumannii* ที่แยกได้จากสิ่งส่งตรวจทางคลินิกของผู้ป่วยในโรงพยาบาลสรรพสิทธิประสงค์ แบคทีเรีย *A. baumannii* มีอัตราการดื้อยาสูงต่อยาต้านจุลชีพที่ใช้บ่อย นอกจากนี้ตรวจพบการสร้างเอนไซม์คาร์บาพีนีเมสกลุ่ม OXA-23 ในแบคทีเรีย *A. baumannii* ที่ดื้อยากลุ่มคาร์บาพีเนม

คำสำคัญ: *Acinetobacter baumannii* ที่ดื้อยากลุ่มคาร์บาพีเนม, เอนไซม์คาร์บาพีนีเมส, ยีน *bla*$_{OXA-23}$

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Abstract

Detection of carbapenemase and *bla*$_{OXA-23}$-like gene in carbapenem-resistant *Acinetobacter baumannii* at Sunpasitthiprasong Hospital

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Introduction: *Acinetobacter baumannii* is a gram negative bacterium causing nosocomial infection resulting in a high prevalence of antibiotic resistances. An increase in the prevalence of carbapenem-resistant *A. baumannii* (CRAB) has been reported worldwide. The objectives of this study were to investigate the antimicrobial resistance rates in clinical isolates of *A. baumannii* isolated from in-patients at Sunpasitthiprasong Hospital and to examine the occurrence of OXA-23 like carbapenemase among the CRAB isolates. **Materials and Methods:** The antimicrobial susceptibility of 33 *A. baumannii* clinical isolates was tested by agar disk diffusion. Fourteen isolates of CRAB were selected and subjected in detection of carbapenemase production by modified Hodge test. The occurrence of *bla*$_{OXA-23}$-like gene in CRAB isolates was detected by polymerase chain reaction (PCR). The amplified fragment was purified and subjected to DNA sequencing. **Results:** Sixteen of thirty-three *A. baumannii* isolates (48.5%) were carbapenem resistance. Imipenem and meropenem resistance rates were 45.2% and 61.5%, respectively. Carbapenemase-producing isolates revealed by modified Hodge test was 71.4% (10 of 14). Out of 14 CRAB isolates, the *bla*$_{OXA-23}$-like gene was detected in the majority (85.7%). **Conclusion:** This study reported data on antimicrobial resistance rate of clinical isolates of *A. baumannii* isolated at Sunpasitthiprasong Hospital. *A. baumannii* showed a high rate of antimicrobial resistance to commonly used antibiotics. In addition, the occurrence of OXA-23 like carbapenemase-producing strains among CRAB isolates was demonstrated.

Keywords: Carbapenem-resistant *Acinetobacter baumannii*, Carbapenemase, *bla*$_{OXA-23}$

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Introduction

Acinetobacter baumannii is a gram-negative coccobacillus causing respiratory and urinary tract infections, as well as meningitis, endocarditis, burn infections, and wound sepsis, especially in intensive care units (ICU) (Bergogne-Bérézin and Towner, 1996; Fournier *et al*., 2006). Multidrug-resistant *A. baumannii* (MDRAB) is emerging as an important nosocomial pathogen in hospital outbreaks (Gordon *et al*., 2010). Carbapenems are usually the antimicrobial agents of choice for treatment of serious infections caused by multidrug-resistant *A. baumannii* (Maragakis *et al*., 2008; Munoz-Price *et al*., 2008). Recently, carbapenem-resistant *A. baumannii* (CRAB) isolates have been increasingly reported

worldwide (Afzal-Shah *et al*., 1998; Poirel *et al*., 2006; Zarrilli *et al*., 2009). In Thailand, many healthcare settings have reported the increasing prevalence of CRAB isolates collected from hospitalized patients. Thapa *et al*. (2010) investigated the mechanism of carbapenem resistance in *A. baumannii* isolated from the patients at Siriraj Hospital, Mahidol University, Bangkok. The results indicated that the CRAB isolates were oligoclonal and the carbapenem resistance was conferred by the presence of bla_{OXA-23} among these isolates. Dejsirilert *et al*. (2009) also reported that a prevalence of imipenem-resistant *A. baumannii* has been continuously rising. Moreover, the resistance rate of *A. baumannii* to imipenem in the Northeastern region was dramatically increased from 2.6% to 60.2% during the year 2000 to 2005. In addition, the results also indicated that the Northeastern region had the highest prevalence of imipenem-resistant *A. baumannii* in 2005. The data from an annual report of antimicrobial susceptibility 2006 at Sunpasitthiprasong Hospital (Ubon Ratchathani province) also revealed the high resistance rate of *A. baumannii* to carbapenems with the percentage of susceptibility to imipenem and meropenem of 28.9 and 43.4, respectively (Sunpasitthiprasong Hospital, 2006). Since the production of carbapenemase is the most important mechanism responsible for carbapenem resistance, one of the most widespread carbapenemases in *A. baumannii* is a class D serine-carbapenemase called OXA-type carbapenemase (Opazo *et al*., 2012; Zarrilli *et al*., 2009). Niumsup *et al*. (2009) as a pioneer also reported an outbreak of CRAB producing OXA-23 carbapenemase isolated from a regional hospital in the north of Thailand. Information and knowledge of the susceptibility patterns and underlying resistance mechanisms of endemic CRAB isolates is essential to set up an appropriate plan for treatment and prevention of the spread of these strains in healthcare settings. However, little is known about the occurrence and underlying resistance mechanisms of these clinical isolates at Sunpasitthiprasong Hospital and other healthcare settings in the south of North-Eastern Thailand.

The objectives of this study were to investigate the antimicrobial resistance rates in clinical isolates of *A. baumannii* isolated from in-patients at Sunpasitthiprasong Hospital and to examine the occurrence of OXA-23 like carbapenemase among CRAB isolates. The antimicrobial susceptibility patterns obtained and the underlying resistance mechanisms of CRAB isolates would benifit for further control and treatment of this highly resistant pathogen.

Materials and Methods

1. Bacterial isolates

A. baumannii ATCC 19606 DMST 10437 was purchased from the Department of Medical Sciences, Thailand-Culture Collection (DMST-CC). *Escherichia coli* ATCC 25922 was obtained from the microbiology laboratory, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University. A total of 33 non-repetitive, clinical isolates of *A. baumannii* were collected between June and September 2008 from in-patients at Sunpasitthiprasong Hospital, Ubon Ratchathani (Table 1). These isolates were obtained from different patients admitted to the intensive care unit (ICU), cardiac care unit (CCU), and the general wards such as medicine, urology, surgery, and pediatric. The sources of the 33 isolates were from several clinical specimens, including blood, urine, tissue, sputum, pus, catheter, and peritoneal fluid. The specimens were aseptically collected by standard procedures with appropriate handling to avoid contamination (Miller *et al*., 2003). All specimens were screened for true infection and the specimens that were considered as colonization were rejected for further identification and antimicrobial susceptibility test. *A. baumannii* was identified by standard biochemical tests (Hall, 2007; Schreckenberger *et al.,* 2003). *A. baumannii* ATCC 19606 DMST 10437 was used as reference control for bacterial identification.

Table 1. Results of antimicrobial susceptibility testing of 33 *A. baumannii* isolates

Isolate	Specimen	AMP	CN	SXT	CXM	FOX	CAZ	CRO	AMC	CIP	IPM	MEM
AB2	Sputum	R	R	R	R	R	R	R	R	S	R	ND
AB7	Sputum	R	S	S	R	R	R	R	S	S	S	ND
AB14	Tissue	R	I	R	R	R	R	R	R	R	S	ND
AB15	Tissue	R	R	R	R	R	R	R	R	R	R	ND
AB16	Urine	R	R	R	R	R	R	R	S	S	S	ND
AB17	Urine	R	R	R	R	R	R	R	R	R	R	R
AB19	Blood	R	R	R	R	R	R	R	R	R	R	R
AB20	Tissue	R	R	S	R	R	R	R	R	R	R	ND
AB23	Blood	R	R	R	R	R	R	R	R	R	S	S
AB27	Tissue	R	S	S	I	I	S	I	S	S	S	ND
AB28	Pus	R	I	R	I	R	S	I	S	S	S	ND
AB29	Sputum	R	I	S	R	R	S	R	R	S	R	ND
AB30	Blood	R	S	S	I	R	S	I	S	S	S	S
AB31	Sputum	R	R	R	R	R	R	R	R	R	R	R
AB32	Sputum	R	R	I	R	R	R	R	R	R	ND	R
AB39	Blood	R	S	S	I	R	S	I	S	S	S	ND
AB41	PD fluid	R	S	S	I	R	I	I	I	S	S	ND
AB43	Tissue	R	R	R	R	R	R	R	R	R	R	R
AB46	Urine	R	R	R	R	R	R	R	S	R	S	ND
AB47	Blood	R	S	S	R	R	S	I	R	S	S	S
AB50	Catheter	R	R	R	R	R	R	R	R	S	R	ND
AB52	Blood	R	R	R	R	R	R	R	R	R	S	S
AB53	Blood	R	S	S	R	R	S	I	I	S	S	S
AB55	Pus	R	R	R	R	R	R	R	R	R	R	ND
AB59	Pus	R	R	R	R	R	R	R	R	R	R	ND
AB61	Urine	R	I	R	R	R	R	R	R	R	R	R
AB62	Blood	R	R	R	R	R	R	R	I	S	S	ND
AB63	Blood	R	S	S	I	I	S	I	S	S	S	ND
AB64	Urine	R	R	R	R	R	R	R	R	R	S	ND
AB71	Urine	R	R	R	R	R	R	R	R	R	ND	R
AB73	Urine	S	S	R	S	S	S	S	S	R	S	ND
AB74	Blood	R	R	R	R	R	R	R	R	R	R	ND
AB75	Blood	R	R	R	R	R	R	R	R	R	R	R

AB: *Acinetobacter baumannii*, PD: peritoneal dialysis, AMP: ampicillin, CN: gentamicin, SXT: co-trimoxazole, CXM: cefuroxime, FOX: cefoxitin, CAZ: ceftazidime, CRO: ceftriaxone; AMC: amoxycillin/clavulanic acid, CIP: ciprofloxacin, IPM: imipenem, MEM: meropenem; S: susceptible, I: intermediate, R: resistant, ND: not detected

2. Antimicrobial susceptibility testing by agar disk diffusion method

Antimicrobial susceptibility was tested by the disk diffusion method. Briefly, the bacterial strains were inoculated onto Muller Hinton Agar (MHA) plates using a sterile cotton swabs. The antibiotic disks were put on the surface of an inoculated MHA and incubated at 35°C. The diameter of an inhibition zone was measured. Antimicrobial susceptibility was interpreted as per the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2008).

3. Detection of carbapenemase by modified Hodge test

Detection of carbapenemase production in CRAB isolates was performed by the modified Hodge test (Lee *et al*., 2001). *Escherichia coli* ATCC 25922, at a turbidity 1/10 of 0.5 McFarland standard solutions, was used to swab onto the surface of a Mueller-Hinton agar plate. A 10 µg imipenem (IPM) disk was placed at the center of an inoculated MHA plate. The test strain was streaked from the center to the plate periphery. The plate was incubated overnight at 37°C. The presence of a distorted inhibition zone was interpreted as a positive result for carbapenemase production screening. An undistorted zone of inhibition was considered as negative (Figure 1).

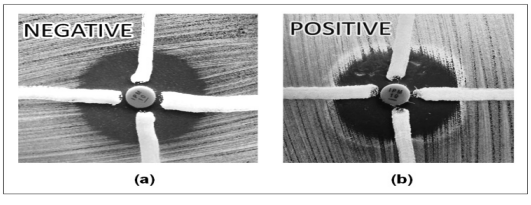

Figure 1. Result of carbapenemase detection by the modified Hodge test. The negative strain shows an undistorted zone of inhibition (a). The presence of a 'cloverleaf-shaped' zone of inhibition was interpreted as a positive result for carbapenemase production (b).

4. PCR amplification of *bla*$_{OXA-23}$-like gene and DNA sequencing

The *bla*$_{OXA-23}$-like gene was amplified by PCR using the OXA-F (5'-TCTGGTTGTACG-GTTCAGC-3') as a forward primer and OXA-R (5'-AGTCTTTCC AAAAATTTTG-3') as a reverse primer (Hujer *et al*., 2006). Both primers were synthesized by BioDesign Co., Ltd., Thailand. A 1:2 dilution of an over-night culture of *A. baumannii* was boiled for 10 min. After centrifugation, the supernatant was collected for use as a DNA template. PCR amplification was performed in a final volume of 20 µl containing reaction buffer 1X, 2mM dNTP, 200 ng forward primer, 200 ng reverse primer, 1.25 U *Taq* polymerase and 4 µl of the DNA template. The PCR reaction was performed using GeneAmp® PCR System 2700 (Applied Biosystems, USA) with a pre-heat at 95°C for 4 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 45 °C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 5 min. PCR products were resolved on 1.2% agarose gels, stained with ethidium bromide, and photographed with a UV transiluminator. The products obtained were presumptive positives based on amplicon size. The PCR product of the isolate AB15 was purified using PCR clean-up Gel extraction NucleoSpin® Extract II (Macherey-Nagel GmbH & Co. KG, Germany) and subjected to DNA sequencing in both directions at BioDesign Co., Ltd., Thailand. Similarity searching and alignment of the obtained nucleotide sequences were performed using the BLAST program available at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Results

1. Antimicrobial susceptibilities

The 33 clinical isolates of *A. baumannii* collected from in-patients at Sunpasitthiprasong Hospital were subjected to antimicrobial susceptibility testing by the agar disk diffusion method according to the criteria of the Clinical and Laboratory Standards Institute (CLSI,

2008). Table 1 shows the antimicrobial susceptibility of the 33 *A. baumannii* isolates. The results revealed that most of the isolates were multidrug resistant *A. baumannii* (MDRAB) strains that confer resistant to three or more than three drugs or drug classes of therapeutic relevance. The antimicrobial susceptibility patterns of *A. baumannii* isolates are summarized in Table 2. The antimicrobial susceptibility testing of the 33 clinical isolates of *A. baumannii* revealed a high resistance rate to 11 antimicrobials tested ranging from 45.2 to 97.0%. The most effective antibiotic against *A. baumannii* isolates was carbapenems (imipenem or meropenem) with a susceptibility rate of 51.5% (17 of 33). Imipenem and meropenem resistance rates were 45.2% and 61.5%, respectively. The second most effective antibiotic against *A. baumannii* isolates was ciprofloxacin with a susceptibility rate 42.4% (14 of 33). Among the 33 *A. baumannii* isolates, 16 (48.5%) were CRAB that also conferred resistance to ampicillin, cefuroxime, cefoxitin, ceftriaxone, and amoxicillin/clavulanic acid.

2. Detection of carbapenemase production and *bla*$_{OXA-23}$-like gene

Fourteen isolates of CRAB were selected and subjected for detection of carbapenemase production and *bla*$_{OXA-23}$-like gene. The results of phenotypic detection of carbapenemase production and PCR detection of *bla*$_{OXA-23}$-like gene are summarized in Table 3. Among the 14 CRAB isolates, 10 (71.4%) were detected as carbapenemase-producing strains by the modified Hodge test.

Table 2. Antimicrobial susceptibility patterns of *A. baumannii* isolates

Antibiotics	Susceptibility pattern (No. of isolates)			% of resistant isolate
	Susceptible (S)	Intermediate (I)	Resistant (R)	
Ampicillin	1	0	32	97.0
Gentamicin	9	4	20	60.6
Co-trimoxazole	10	1	22	66.7
Cefuroxime	1	6	26	78.8
Cefoxitin	1	2	30	90.9
Ceftazidime	9	1	23	69.7
Ceftriaxone	1	8	24	72.7
Amoxicillin/clavulanic acid	9	3	21	63.6
Ciprofloxacin	14	0	19	57.6
Imipenem	17	0	14	45.2
Meropenem	5	0	8	61.5

Table 3. Results of detection of carbapenemase and bla_{OXA-23} like gene

Bacteria	Carbapenemase	*bla* OXA-23 -like gene
ATCC 19606	ND	-
AB2	+	-
AB15	+	+
AB17	-	+
AB19	-	+
AB20	+	+
AB29	+	+
AB31	-	+
AB43	+	+
AB50	+	+
AB55	+	+
AB59	+	+
AB61	+	+
AB74	+	-
AB75	-	+
No. of positive (%)	**10 (71.4)**	**12(85.7)**

ND: not detected

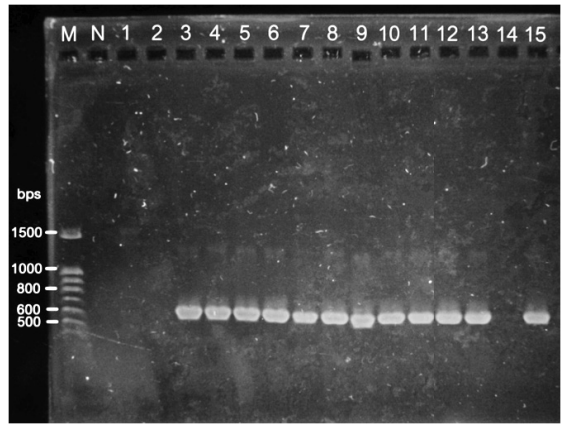

Figure 2. PCR amplification of bla_{OXA}-like gene. M: 100 bp DNA Ladder, N: Negative, 1: ATCC 19606 , 2: AB2, 3: AB15, 4: AB17, 5: AB19, 6: AB20, 7: AB29, 8: AB31, 9: AB43, 10: AB50, 11: AB55, 12: AB59, 13: AB61, 14: AB74, 15: AB75

The bla_{OXA-23}-like gene is one of the common genes encoding for OXA-carbapenemase that has been detected throughout the world (Amudhan *et al.,* 2011; Mugnier *et al.,* 2010). The occurrence of this gene in CRAB isolated in this study was detected by PCR amplification using OXA-F/OXA-R primers. This primer set was designed to amplify a part of bla_{OXA-23}-like gene (Hujer *et al*., 2006). The size of PCR product amplified by OXA-F/OXA-R primer set was approximately 600 bp as expected (Figure 2). The bla_{OXA-23}-like gene was found in the majority of CRAB isolates (12 /14, 85.7%). A sequence analysis of the amplified fragment of the isolate AB15 confirmed the presence of bla_{OXA-23} gene (Figure 3). A homology search using *blastn* algorithm revealed that the amplified fragment has a high similarity with several sequences of class D beta-lactamase OXA-23 gene from *A. baumannii* deposited in GenBank (92% identity).

5'- TGGTNGTAATGTGAATTGTGATATACTCCCCCCCTGANGTGTTCATGGACNTTGTGATGTT CATAATT-GGGGNATCC ATGTAAAATTTTCATGGTGTGTGTATGTTTCAATGTCCATNAATTAAATCCCTGTTGTTGGNAAT-GTGGATATTGATACCATACCTTTCGAANGGTTTGTTTGGTTCTTGTTGTGTTTTAATTTTTCCAGATACCAG-GGATGTAGTTTTATAAAGTTTTGNGATTTTCNTTAATATTTGGTGGTAAAATTGGGCCGTGCTGTAAAANGTC-CCCCGGNNGGGCTAAAATTAACTAAAGGGANTAATTAAATTGNGTTGCCCCGAGGCCCTCTTTTAAC-CAGTGTTTGAAAAATTGTTTGGAAAATTGTCCTTGAATTCGGATTGGAGAACCAGTNAAAACGGATATTAAT-GGAAATATTTAAAATGGAAAGGGCGAGANAATGGTCATTTACCGCTTGGGAAANAAGACATGACTACTAG-GAGAAGCCATGAAGCTTGTCTGCAGTCCCAGTCTATTCAGGAACGTGCNCGACGTATCGGTCTTGATCTCAT-GCAAAAAGAAGTATAAACGTATTGGTGTTCGGTAATGCTGAAATTGGACAGCAGGTTGATAATTTCTGGTGT-GGTAGGACCATTAAAGTGTACGCCTATTTCAAGAGTAGAGTTGTTTTCCCNATTTAGCACATGACACAGCT-TCCATTAGTGATAAGTGCGCTANTTTTTTGN **-3'**

Figure 3. DNA sequence of the *bla*$_{OXA-23}$-like gene amplified from the isolate AB15

Discussion and Conclusion

The high prevalence of CRAB has been increasingly reported worldwide (Al-Sweih *et al*., 2012; Chen *et al*., 2009; Kulah *et al*., 2010; Routsi *et al*., 2010). In Thailand, Chaiwarith *et al*. (2005) revealed that less than 40 percent of *A. baumannii* isolates obtained from patients admitted to Maharaj Nakorn Chaing Mai Hospital during July and October 2003 were susceptible to imipenem and meropenem. Similarly, at Siriraj Hospital in 2002, the susceptibility of *A. baumannii* to carbapenems was 32% (Keerasuntonpong *et al*., 2006). In addition, the percentage of CRAB was found to be 84.2% of the *A. baumannii* isolated from patients at Phramongkutklao Hospital, Bangkok in which all isolates were susceptible only to colistin and tigecycline (Aimsaad *et al*., 2009). The results of antimicrobial susceptibility test emphasized that there are limited antibiotics available for the treatment of CRAB infections (Karageorgopoulos *et al.,* 2008; Neonakis *et al.,* 2011). Therefore, the prevention of the spread of this highly resistant pathogen is greatly necessary. Although the percentage of CRAB found in this study was relatively lower than those reported from other hospitals, it would not probably imply that the prevalence of CRAB found at Sunpasitthiprasong Hospital is different from other hospitals, and not a critical case for this pathogen infection. But one should be concerned that if a CRAB outbreak really happened in this region, how the therapeutic treatments for *A. baumannii* infection would be addressed.

The *bla*$_{OXA-23}$-like gene is a common type of OXA carbapenemase contributing to carbapenem resistance in clinical isolates of *A. baumannii*. This type of resistance is a significant threat in hospitals (Amudhan *et al*., 2011; Mugnier *et al*., 2010). The high prevalence of *A. baumannii* harboring *bla*$_{OXA-23}$-like gene in Thailand has been also reported previously (Niumsup *et al*., 2009; Thapa *et al*., 2010). However, this is the first study and report of the occurrence of CRAB isolates that produce carbapenemase and carry *bla*$_{OXA-23}$-like gene at Sunpasitthiprasong Hospital, a tertiary healthcare center in the south of North-East Thailand. The results obtained in this study suggest that OXA-23 like carbapenemase may be an important contributory mechanism for the carbapenem resistance among CRAB isolates presenting at Sunpasitthiprasong Hospital. However, the exact mechanisms contributing to carbapenem resistance in these isolates should be further elucidated. For example, further studies are required to investigate the presence of other possible genes encoding carbapenemases such as Ambler class B genes that encode for metallo-ß-lactamases (Opazo *et al*., 2012). Moreover, non-enzymatic

mechanisms, including changes in outer-membrane proteins, multidrug efflux pumps, and alteration in the affinity or expression of penicillin-binding proteins associated with carbapenem resistance in *A. baumannii* (Peleg *et al*., 2008) should be also determined. In addition, the effective antibiotics for the resistance strains need to be investigated.

In conclusion, the present study reported data on antimicrobial resistance rate of clinical isolates of *A. baumannii* isolated at Sunpasitthiprasong Hospital. *A. baumannii* isolates showed a high percentage of multidrug resistance to commonly used antibiotics. In addition, this study also demonstrated the occurrence of carbapenemases among clinical isolates of CRAB at Sunpasitthiprasong Hospital with a high prevalence of bla_{OXA-23} in these isolates.

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