ตรวจสอบเอนไซม์คาร์บีนีเมสและยีนกลุ่ม bla\textsubscript{OXA-23} ที่ติดต่อกับกลุ่มคาร์บีนีเมสในโรคระบาดสตรีทิปัสปอริกolec

ศิริมา สุวรรณกูฏ จันติมา, ธัญพงศ์ คุณะวัฒนกุล, ศราวุฒิ เสมารัมย์, นิตยา ธีระวัฒนสุข จันติมา 1*, ศุภเดช แสงสานนท์, เกรวิทย์ จันติมา 2, ประวิทิป ธรรมวัฒนสุข 3, ว. เกษศักดิ์เศรษฐี 2556; 9(2) : 99-109

Received : 27 December 2012  Accepted : 26 July 2013

บทคัดย่อ

ตรวจสอบเอนไซม์คาร์บีนีเมสและยีนกลุ่ม bla\textsubscript{OXA-23} ในแบคทีเรีย Acinetobacter baumannii ที่ดื้อต่อยากลุ่มคาร์บีนีเมสในโรงพยาบาลสรรพสิทธิประสงค์ ศิริมา สุวรรณกูฏ จันติมา, ธัญพงศ์ คุณะวัฒนกุล, ศราวุฒิ เสมารัมย์, นิตยา ธีระวัฒนสุข, เกรวิทย์ จันติมา 1*.

ว. เกษศักดิ์เศรษฐี 2556; 9(2) : 99-109

บทนำ: Acinetobacter baumannii คือเชื้อแบคทีเรียแกรมลบที่เป็นสาเหตุของการติดเชื้อในโรงพยาบาลที่มีอัตราการติดต่ออยู่ในรูปของรูปปั้น มีรายงานการเพิ่มขึ้นของความชุกของแบคทีเรีย A. baumannii ที่ดื้อต่อยากลุ่มคาร์บีนีเมสทั่วโลก วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อศึกษาอัตราการติดต่อ daklujudชิพของแบคทีเรีย A. baumannii ที่แยกได้จากติ้งสงตรวจทางคลินิกของผู้ป่วยที่โรงพยาบาลสรรพสิทธิประสงค์ จังหวัดอุบลราชธานี และตรวจสอบการสร้างเอนไซม์การดำรงเผิงกลุ่ม OXA-23 ของแบคทีเรีย A. baumannii ที่แยกกลุ่มคาร์บีนีเมใส่ยีนกลุ่ม bla\textsubscript{OXA-23}

วัสดุและวิธีการทดลอง:

ทดสอบความไวต่อยากลุ่มเครื่องมือ A. baumannii จำนวน 33 ไอโซเลตด้วยวิธี agar disk diffusion คัดเลือกแบคทีเรีย A. baumannii ที่มีการไม่ต่อยากลุ่มคาร์บีนีเมสจำนวน 14 ไอโซเลต เพื่อนำไปตรวจสอบการสร้างเอนไซม์การดำรงเผิงเมสแต่ยีน modiﬁed Hodge test ตรวจสอบยีนกลุ่ม bla\textsubscript{OXA-23} นโยบายแบคทีเรีย A. baumannii ที่มีการไม่ต่อยากลุ่มคาร์บีนีเมส

ผลการทดลอง:

การทดสอบความไวต่อยากลุ่มเครื่องมือ แบคทีเรีย A. baumannii จำนวน 16 ไอโซเลต ถือเป็นร้อยละ 48.5 อัตราการติดต่ออยู่ imipenem และ meropenem ทำกับร้อยละ 45.2 และ ร้อยละ 61.5 ตามลำดับ ร้อยละของแบคทีเรียที่สร้างเอนไซม์การดำรงเผิงเมสเท่ากับร้อยละ 71.4 (10 จาก 14) จากการตรวจสอบยีนกลุ่ม bla\textsubscript{OXA-23} นโยบายแบคทีเรีย A. baumannii ที่มีการไม่ต่อยากลุ่มคาร์บีนีเมส จำนวน 14 ไอโซเลต ตรวจพบยีนกลุ่ม bla\textsubscript{OXA-23} ในแบคทีเรีย A. baumannii จำนวน 13 ไอโซเลต พบแบบที่แบคทีเรีย A. baumannii ที่มีการไม่ต่อยากลุ่มคาร์บีนีเมส จำนวน 16 ไอโซเลต คิดเป็นร้อยละ 85.7 สรุปผล: การศึกษาครั้งนี้มีรายงานข้อมูลเพื่อให้ทราบถึงการสร้างเอนไซม์การดำรงเผิงเมสในแบคทีเรีย A. baumannii ที่แยกได้จากติ้งสงตรวจทางคลินิกของผู้ป่วยในโรงพยาบาลสรรพสิทธิประสงค์ แบคทีเรีย A. baumannii มีอัตราการติดต่อ daklujudชิพของแบคทีเรีย Acinetobacter baumannii ที่มีการไม่ต่อยากลุ่มคาร์บีนีเมส, แบคทีเรีย Acinetobacter baumannii OXA-23

บทความที่มีความสำคัญ:

Acinetobacter baumannii ที่ดื้อต่อยากลุ่มคาร์บีนีเมส, เอนไซม์การดำรงเผิงเมส, ยีน bla\textsubscript{OXA-23}

1 อาจารย์ประจุ คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี อ. วารินชําราบ จ. อุบลราชธานี 34190
2 นักศึกษาคณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี อ. วารินชําราบ จ. อุบลราชธานี 34190
3 โรงพยาบาลสรรพสิทธิประสงค์ จ. อุบลราชธานี 34000
4 ผู้ช่วยศาสตราจารย์, นักวิชาศาสตร์การเกษตร มหาวิทยาลัยเทคโนโลยีสุรนารี อ. เมือง จ.นครราชสีมา 30000
5 ติดต่อผู้ช่วยศาสตราจารย์: โทรศัพท์ 045-353617 โทรสาร 045-353626 อีเมล: phsirisu@ubu.ac.th
Abstract

Detection of carbapenemase and bla_{OXA-23}-like gene in carbapenem-resistant Acinetobacter baumannii at Sunpasitthiprasong Hospital

Sirima Suvarnakuta Jantama1*, Tanyapong Kunawatanakul2, Sarawut Semaram2, Suppadet Sangsanon3, Nidtaya Teerawattanasuk3, Kaemwich Jantama4
IJPS, 2013; 9(2) : 99-109

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}

1 Ph.D., Lecturer, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand
2 Pharmacy students, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand
3 M.Sc., Medical Technologist, Clinical Pathology Department, Sunpasitthiprasong Hospital, Ubon Ratchathani 34000, Thailand
4 Ph.D., Assistant Professor, Metabolic Engineering Research Unit, School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand
* Corresponding author: Tel 045-353617, Fax 045-353626, E-mail: phsirisu@ubu.ac.th

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 befit 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   | 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   | S   | ND  |     |
| AB15    | Tissue   | 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   |     |     |
| AB19    | Blood    | R   | R  | R   | R   | R   | R   | R   | R   | R   |     |     |
| AB20    | Tissue   | R   | R  | S   | R   | R   | R   | R   | R   | R   | ND  |     |
| AB23    | Blood    | 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   |     |
| AB29    | Sputum   | R   | I  | S   | R   | R   | S   | R   | S   | R   | ND  |     |
| AB30    | Blood    | R   | S  | S   | I   | R   | S   | I   | S   | S   | S   |     |
| AB31    | Sputum   | R   | R  | R   | R   | R   | R   | R   | R   | R   |     |     |
| AB32    | Sputum   | R   | R  | I   | R   | R   | R   | R   | R   | R   | ND  |     |
| AB39    | Blood    | R   | S  | S   | I   | R   | S   | I   | S   | S   | ND  |     |
| AB41    | PD fluid | R   | S  | S   | I   | R   | I   | I   | I   | S   | S   |     |
| AB43    | Tissue   | R   | R  | R   | R   | R   | R   | R   | R   | R   | R   |     |
| AB46    | Urine    | 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   | S   | S   |     |
| AB53    | Blood    | R   | S  | S   | R   | R   | S   | 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   |     |
| AB62    | Blood    | R   | R  | R   | R   | R   | R   | R   | R   | R   |     |
| 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   | ND  | R   |     |
| AB73    | Urine    | S   | S  | R   | S   | S   | S   | S   | S   | S   | 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   | ND  |

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).

![NEGATIVE](image1.png) ![POSITIVE](image2.png)

**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\text{\textsubscript{OXA-23}}-like gene and DNA sequencing

The bla\text{\textsubscript{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 overnight 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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Susceptibility pattern (No. of isolates)</th>
<th>% of resistant isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible (S)</td>
<td>Intermediate (I)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Imipenem</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Meropenem</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>
The \textit{bla}_{\text{OXA-23}}-like gene is one of the common genes encoding for OXA-carbapenemase that has been detected throughout the world (Amudhan \textit{et al.}, 2011; Mugnier \textit{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 \textit{bla}_{\text{OXA-23}}-like gene (Hujer \textit{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 \textit{bla}_{\text{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 \textit{bla}_{\text{OXA-23}} gene (Figure 3). A homology search using \textit{blastn} algorithm revealed that the amplified fragment has a high similarity with several sequences of class D beta-lactamase OXA-23 gene from \textit{A. baumannii} deposited in GenBank (92% identity).

### Table 3. Results of detection of carbapenemase and \textit{bla}_{\text{OXA-23}}-like gene

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Carbapenemase</th>
<th>\textit{bla}_{\text{OXA-23}}-like gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 19606</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>AB2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>AB15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AB17</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AB19</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AB20</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AB29</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AB31</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AB43</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AB50</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AB55</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AB59</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AB61</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AB74</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AB75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No. of positive (%) 10 (71.4) 12 (85.7)

ND: not detected

Figure 2. PCR amplification of \textit{bla}_{\text{OXA-23}}-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
Detection of carbapenemase and bla\textsubscript{OXA-23}-like gene in carbapenem-resistant Acinetobacter baumannii at Sunpasitthiprasong Hospital

Jantama S. S. et al.

Vol. 9 No. 2 May-August 2013

Figure 3. DNA sequence of the bla\textsubscript{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 Chiang 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\textsubscript{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\textsubscript{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\textsubscript{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-\beta-lactamas (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 \emph{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 \emph{A. baumannii} isolated at Sunpasitthiprasong Hospital. \emph{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 \textit{bla}_{OXA-23} in these isolates.

**Acknowledgements**

This work was partially supported by a Young Researchers Grant from Ubon Ratchathani University. The authors thank the Faculty of Pharmaceutical Sciences, Ubon Ratchathani University for providing research facilities. Thanks also to Bob Tremayne, Division of International Relations, Ubon Ratchathani University for assistance with English.

**References**


Hujer KM, Hujer AM, Hulten EA, \textit{et al.} Analysis of Antibiotic Resistance Genes in Multidrug-
Detection of carbapenemase and bla\textsubscript{OXA-23}-like gene in carbapenem-resistant Acinetobacter baumannii at Sunpasitthiprasong Hospital

Jantama S. S. et al.

Resistant Acinetobacter sp. Isolates from Military and Civilian Patients Treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 2006; 50(12): 4114-4123.


Sunpasitthiprasong Hospital, Clinical Pathology Department. Annual report of antimicrobial susceptibility. Ubon Ratchathani: Sunpasitthiprasong Hospital; 2006.