Identification of *bla*OXA23 and *bla*NDM1 from Carbapenem-resistant *Acinetobacter baumannii* at a Private Hospital in Thailand

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**Abstract**

**OBJECTIVES:** This study aimed to detect carbapenemase genes and their clonal relationships among carbapenem-resistant *Acinetobacter baumannii* (CRAB) clinical isolates.

**MATERIAL AND METHODS:** Fifteen CRAB isolates were collected from patients admitted to Phyathai II International Hospital, Bangkok, Thailand during August 2014 – April 2015. Polymerase chain reaction (PCR) amplification and DNA sequencing were used to identify *bla*OXA23, *bla*OXA40, *bla*OXA48, *bla*OXA58, *bla*IMP, *bla*VIM, *bla*KPC, and *bla*NDM. Clonal relationships were explored by using repetitive element palindromic (REP)-PCR.

**RESULT:** The CRAB isolates were categorized by REP-PCR in 8 groups [A-H], with 53.3% belonging to group A, whereas the remaining 7 clones were in each member of B-H, respectively. The *bla*OXA23 was detected in most CRAB isolates (86.7%) whereas only two isolates harbored *bla*NDM with *bla*OXA23 (13.3%).

**CONCLUSION:** Most CRAB strains carried *bla*OXA23 as reported in several related studies but our finding confirmed the emergence of CRAB carrying multiple types of carbapenemase genes in Thailand. This is a worrying phenomenon that concerns the spread of such CRAB genotypes.

**Keywords:** OXA carbapenemase, carbapenemase, metallo-beta lactamase, clonal relationship

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In Thailand, several reports have revealed that bla\textsubscript{OXA23} constituted the majority of carbapenemase genes\textsuperscript{6,7} but the rare prevalence of OXA-40\textsuperscript{8} and IMP-1\textsuperscript{9} among CRAB clinical isolates was observed. However, previous studies were conducted in university hospitals and a general hospital. The resistant mechanisms in CRAB from a private hospital might be different regarding the patterns of antibiotic use, patient characteristics and infection prevention and control methods. Thus, our study aimed to identify the presence of carbapenemase genes and clonal relationship among CRAB strains isolated from patients admitted to a private hospital.

**Materials and Methods**

**Bacterial strains**

All clinical \textit{A. baumannii} strains were obtained from patients admitted to Phyathai II International Hospital, a 550-bed private hospital between August 2014 and April 2015. A carbapenem resistant strain was defined as isolates that were phenotypically resistant to imipenem (10 µg) and meropenem (10 µg) using the disk diffusion method based on The Clinical and Laboratory Standards Institute (CLSI).\textsuperscript{10} Only the first CRAB isolate from each patient was kept in tryptic soy broth containing 20% glycerol at -80°C until studied. The research protocols were approved by the Ethic Committee [No. ID0014/59].

**Carbapenem genes**

Each DNA sample of CRAB strains was extracted using a commercial kit (RBC Bioscience, California, USA). The primers of genes (bla\textsubscript{OXA23}, bla\textsubscript{OXA40} and bla\textsubscript{OXA58}) and conditions that were used, are described in Table 1. Thermocycler was performed as follows: 94°C for 5 minutes; 30 cycles of 94°C for 45 seconds, annealing temperature specific for each primer pair for 45 seconds, and 72°C for 1 minute; with a final heating at 72°C for 10 minutes.\textsuperscript{8}

The detection of carbapenemase genes including bla\textsubscript{IMP}, bla\textsubscript{VIM}, bla\textsubscript{KPC}, bla\textsubscript{OXA48} and bla\textsubscript{NDM} was performed, however, with multiplex PCR (Table 1). Amplification was carried out with the following thermal cycling conditions: at 94°C for 10 minutes and 36 cycles of amplification consisting of 30 seconds at 94°C, 40 seconds at 52°C, and 50 seconds at 72°C, with 5 minutes at 72°C for the final extension.\textsuperscript{11}

All amplicons were separated by agarose gel electrophoresis, stained with ethidium bromide, and compared with those of known carbapenemase genes. Finally, their identities were confirmed by nucleotide sequencing (Ward Medic, Ltd, Bangkok, Thailand) and were compared with known sequences in the GenBank database.

**Table 1**: Primers, amplicon sizes and annealing temperature used in PCR-based detection of \textit{A. baumannii} carbapenemase genes.\textsuperscript{8,11}

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Size of amplicon (bp)</th>
<th>Annealing temperature (°C)</th>
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</thead>
</table>
| bla\textsubscript{OXA23} | F- 5’ GGAATTCCATGAATAAATATTTA 3’  
R-5’ GGATCCGGTTAAATATTTAGCAG 3’ | 822 | 42 |
| bla\textsubscript{OXA40} | F-5’ GGAATTCCATGAAAAATTTATAC 3’  
R-5’ GGATCCGGTTAAATGATTCCAAGA 3’ | 828 | 45 |
| bla\textsubscript{OXA58} | F-5’ GGAATTCCATGAATATTATTGATAA 3’  
R-5’ GGATCCGGTTAAAAATATGAA 3’ | 843 | 45 |
| bla\textsubscript{IMP} | F-5’ GGAATTACATGAAAAATTATTAC 3’  
R-5’ GGATCCGGTTAAATGATTCCAAGA 3’ | 843 | 45 |
| bla\textsubscript{VIM} | F-5’ GGAATTACATGAAAAATTATTAC 3’  
R-5’ GGATCCGGTTAAATGATTCCAAGA 3’ | 843 | 45 |
| bla\textsubscript{KPC} | F-5’ GGAATTACATGAAAAATTATTAC 3’  
R-5’ GGATCCGGTTAAATGATTCCAAGA 3’ | 843 | 45 |
| bla\textsubscript{OXA48} | F-5’ GGAATTACATGAAAAATTATTAC 3’  
R-5’ GGATCCGGTTAAATGATTCCAAGA 3’ | 843 | 45 |
| bla\textsubscript{NDM} | F-5’ GGAATTACATGAAAAATTATTAC 3’  
R-5’ GGATCCGGTTAAATGATTCCAAGA 3’ | 843 | 45 |

**Clonal relationships**

The clonal relationships of CRAB were evaluated using the REP-PCR method. The 15 µl PCR mixture was composed of 1 µl of DNA, 0.4 µl of 20 µM each forward and reverse primers, 7.5 µl of PCR master mix kit (JumpStart Red Taq\textsuperscript{8} Ready Mix, California, USA) and 5.7 µl of DNAase-free water. A couple primer (REP-forward: 5’-IIIGC GCCGICAT-CAGGC-3’ and REP-reverse: 5’-ACGTCTTATCAG-GCCTAC-3’) was used to amplify the REP region under the following conditions, starting with heating at 94°C for 10 minutes, followed by 30 cycles of 94°C for 1 minute, 45°C for

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\textsuperscript{8} Ready Mix, California, USA
1 minute, 72°C for 2 minutes and finally at 72°C for 16 minutes. The REP-PCR products were performed using agarose gel electrophoresis and were stained with ethidium bromide. The criterion for classifying the different clones was a pattern that differed from the at least three bands or more of REP-PCR. 

**Results**

Over the nine-month study period, only the first CRAB strain isolated from each patient, for a total of 15 clinical isolates were determined. The 15 samples were isolated from sputum (n = 9), blood (n = 2), urine (n = 1), pus (n = 2) and tissue (n = 1) specimens. All CRAB strains resisted to ceftazidime and were susceptible to ciprofloxacin, amikacin, and ampicillin/sulbactam at 6.7%, 26.7%, and 26.7%, respectively.

According to the clonal relationship study, the CRAB isolates were categorized by REP-PCR in 8 groups [A-H], with 53.3% belonging to group A, whereas the remaining 7 clones were in each member of B-H, respectively. (Figure 1). Of the OXA and MBL genes identified, most CRAB carried only *blaOXA23* (86.7%) whereas only two isolates harbored both *blaNDM1* and *blaOXA23* (13.3%) (Figure 2). However, no *blaOXA40*, *blaOXA58*, *blaIMP*, *blaVIM*, and *blaKPC* were identified in the study.

![Figure 1A-B: Pattern obtained with Repetitive Extragenic Palindromic-Polymerase Chain Reaction (REP-PCR), The letters above each lane indicate the strain (No 1-15); Ladder, DNA molecular weight marker in kilobase unit (kb); Neg, Negative control. Using the REP-PCR method, there were 8 groups divided into patterns A-E (1A) and patterns F-H (1B).](image)

![Figure 2: PCR detection of presence of NDM-1 gene in *Acinetobacter baumannii* isolate No 3 and 4. Ladder, molecular size markers (size (bp) is indicated in the left margin); Neg, negative control; IMP positive control; VIM positive control; OXA-48 positive control; NDM positive control; KPC positive control; 1-10, test samples.](image)
Discussion

Currently, with the mechanisms of resistance in *A. baumannii* especially, carbapenemases has been reported from various parts of the world. Although six studies detected carbapenemase genes in Thailand only five were from the clinical isolates in the university hospitals and the remaining were from a general hospital.1,4,11–14 Actually, the diversity of resistant mechanisms among *A. baumannii* clones might be possible in different hospitals.15 Moreover, at the same hospital but in different wards, the distribution of clones and mechanism of resistance also varied in different clinical departments.16 Thus, our study, performed in a private hospital, revealed the same *bla*$_{OXA23}$ as related studies but reported two patients carrying two carbapenemase genes (*bla*$_{OXA23}$ and *bla*$_{NDM}$) simultaneously.

With the NDM-1, the Amber class B, MBL group, is one of the most commonly reported among *Enterobacteriaceae*, being firstly identified in a patient who had returned from New Delhi.17 *BlaNDM*-carrying *Enterobacteriaceae* remains on the Indian subcontinent, but to date, has been found in various parts of the world.17 Of *bla*$_{NDM}$-*bla*$_{OXA23}$ carrying *A. baumannii*, the co-carbapenemase genes found in our study, this phenomena was similar to a related study showing the coexistence of *bla*$_{OXA1}$-*bla*$_{NDM}$ among three isolates of CRAB in India and two isolates of CRAB in Thailand.18,19 However, we could not explain how these genes coexisted. However, we hypothesize that the co-genes might have been transferred by mobile genetic elements within *IS*aba1.18

However, no other carbapenemase genes (*bla*$_{OXA48}$, *bla*$_{KPC}$, *bla*$_{IMP}$ or *bla*$_{VIM}$) were detected in the present study. This might be due to the small sample size, limited period of sample collection, or their extremely low prevalence in the hospital setting. This limitation should be corrected by further studies with a larger sample size and a longer study period.

At the time of writing, avibactam and vaborbactam are diazabicyclo-octane and cyclic boronic acid respectively, having an inhibitor activity against class A, class C and some enzymes in class D beta-lactamases. Whereas class B metallo-beta lactamas (such as NDM, IMP, VIM) have proven to have less inhibition by avibactam and vaborbactam. Thus, the presence of a pathogen carrying co-carbapenemase gene (*bla*$_{OXA23}$ and *bla*$_{NDM}$) is challenging treatment for finding a novel β-lactamase inhibitor with high affinity to all classes of beta-lactamases.20

Regarding the clonal relationships in this study, clone A (53.3%) was predominant. This prevalence of the majority clone was less than that reported in a related study (93.0%).4 The lower prevalence of the predominant clone and the numerous types of clone might have stemmed from well-controlled multiple factors including strict infection control guidelines, appropriate use of antibiotics and notification of infected patients.21 However, as clone A exhibited the highest prevalence, we suggested that the infectious control program could continue minimizing the reservoir for bacterial transmission in the hospital.22

Conclusion

This research comprised a study to confirm the most common type of *bla*$_{OXA23}$ found in Thailand beyond academic medical centers. Additionally, this study firmly showed *bla*$_{NDM}$ coexisted with *bla*$_{OXA23}$ in clinical CRAB isolates in Thailand.

Conflict of Interests

The authors declare no conflict of interest.
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