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_{NDM1} 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

Acinetobacter baumannii, a Gram-negative coccobacilli, is a major causative pathogen involving nosocomial infection in various organs, such as the lower respiratory tract, skin/soft tissue, blood and, rarely, in the urinary tract and central nervous system.[1] In Thailand, regarding the data of the first half-year of 2018,[2] *A. baumannii* was the third and second ranked organism isolated from all specimens and sputum, respectively.

This pathogen is not only an important nosocomial pathogen, but also has multiple mechanisms to resist the current antimicrobials varied from multi-drug treatment (more than three groups), extensive drug resistance (resistant to all antibiotics except colistin and tigecycline) to pan-drug (resistant to all available antibiotics).[3] Over the past 19 years (from 2000-2018), the prevalence of carbepenem resistant *A. baumannii* (CRAB) has increased.[2] The National Antimicrobial Resistance Surveillance, Thailand (NARST) reported that among *A. baumannii* isolates from hospitalized patients in 50 hospitals, the rate of CRAB increased from 5.8% in 2000 to 52.5% in 2018.[2]

However, the increasing rate of CRAB has affected carbapenems use as empirical therapy for infections suspected of *A. baumannii.* The known type of carbapenemase enzyme in CRAB remains important for some circumstances including the role of carbapenems in combination with the other antimicrobials against CRAB and the upcoming use of new beta-lactamase inhibitor such as avibactam against carbapenemase producing organisms.[4,5]

To date, carbapenem-destroying enzymes in CRAB have been found in two major types, namely, OXA-carbapenemases and metallo-beta lactamases.[1]

In Thailand, several reports have revealed that *bla*$_{OXA23}$ constituted the majority of carbapenemase genes[6,7] but the rare prevalence of OXA-40[8] and IMP-1[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 *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).[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].

Carbapenemase genes

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

The detection of carbapenemase genes including *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{KPC}$, *bla*$_{OXA48}$ and *bla*$_{NDM}$ 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.[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 *A. baumannii* carbapenemase genes. [8,11]

Gene	Primer sequence	Size of amplicon (bp)	Annealing temperature (ºC)
bla$_{OXA23}$	F- 5' GGAATTCCATGAATAAATATTTTA 3' R-5' GGATCCCGTTAAATAATATTCAGC 3'	822	42
bla$_{OXA40}$	F-5' GGAATTCCATGAAAAAATTTATAC 3' R-5' GGATCCCGTTAAATGATTCCAAGA 3'	828	45
bla$_{OXA58}$	F-5' GGAATTCCATGAAATTATTAAAAA 3' R-5' GGATCCCGTTATAAATAATGAAAA 3'	843	45
bla$_{IMP}$	F-5' GGAATAGAGTGGCTTAAYTCTC 3' R-5' GGTTTAAYAAAACAACCACC 3'	232	52
bla$_{VIM}$	F-5' GATGGTGTTTGGTCGCATA 3' R-5' CGAATGCGCAGCACCAG 3'	390	52
bla$_{KPC}$	F-5' CGTCTAGTTCTGCTGTCTTG 3' R-5' CTTGTCATCCTTGTTAGGCG 3'	798	52
bla$_{NDM}$	F-5' GGTTTGGCGATCTGGTTTTC 3' R-5' CGGAATGGCTCATCACGATC 3'	621	52
bla$_{OXA48}$	F-5' GCGTGGTTAAGGATGAACAC 3' R-5' CATCAAGTTCAACCCAACCG 3'	438	52

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® 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 94ºC for 10 minutes, followed by 30 cycles of 94ºC for 1 minute, 45ºC for

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1 minute, 72ºC for 2 minutes and finally at 72ºC for 16 minutes [8]. 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.[12]

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 bla_{OXA23} (86.7%) whereas only two isolates harbored both bla_{NDM1} and bla_{OXA23} (13.3%) (Figure 2). However, no bla_{OXA40}, bla_{OXA58}, bla_{IMP}, bla_{VIM}, and bla_{KPC} were identified in the study.

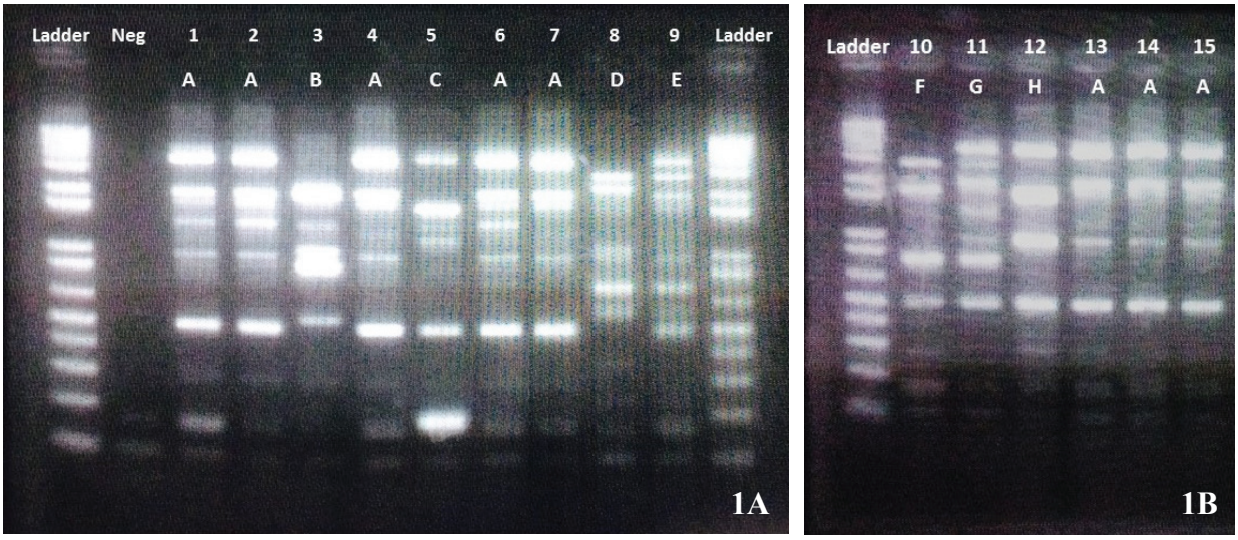

Figure 1A-B: Pattern obtained with Repetitive Extragenic Palindromic-Polymerase Chain Reaction (REP-PCR), The letters above each lane indicate the strain (No 1-15); Ladder, DNA molecular weight marker in kilobase unit (kb); Neg, Negative control. Using the REP-PCR method, there were 8 groups divided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

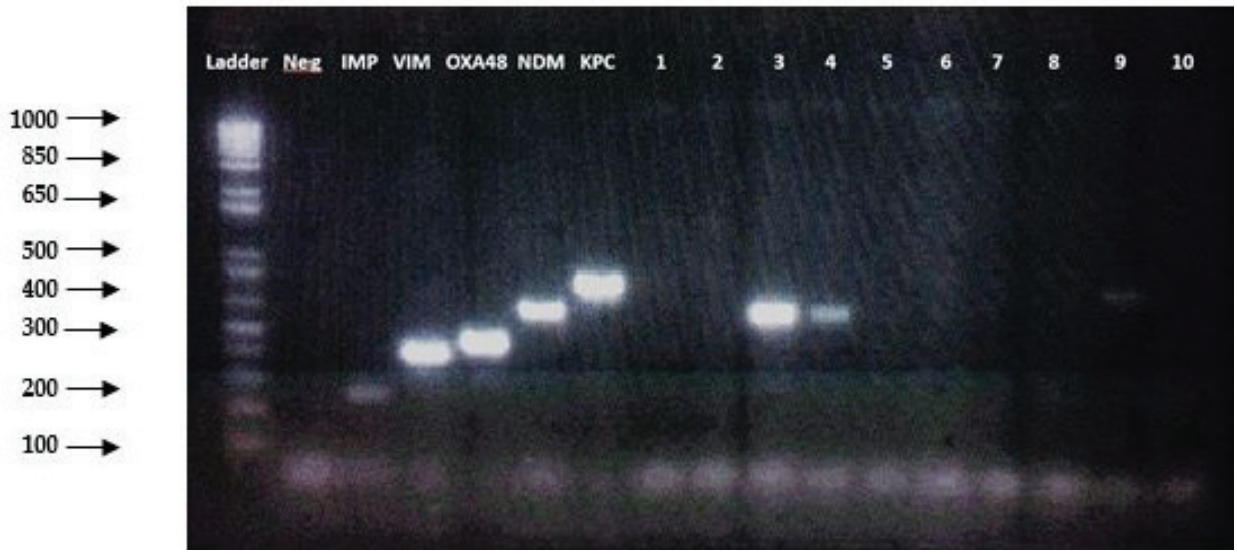

Figure 2: PCR detection of presence of NDM-1 gene in *Acinetobacter baumannii* isolate No 3 and 4. Ladder, molecular size markers (size (bp) is indicated in the left margin); Neg, negative control; IMP positive control; VIM positive control; OXA-48 positive control; NDM positive control; KPC positive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

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. [6-9,13,14] 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_{NDM1}) 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] Bla_{NDM}-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_{OXA}-bla_{NDM1} 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 lactamases (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_{NDM1}) 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%).[8] 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_{NDM1} coexisted with bla_{OXA23} in clinical CRAB isolates in Thailand.

Conflict of Interests

The authors declare no conflict of interest.

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