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Prevalence of *bla*$_{OXA}$ genes in carbapenem-resistant *Acinetobacter baumannii* isolates from clinical specimens from Nopparatrajathanee Hospital

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Abstract

This study aimed to investigate the prevalence of *bla*$_{OXA}$ genes in carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates from Nopparatrajathanee Hospital and the resistance rate against antimicrobial agents used for *A. baumannii* treatment. The susceptibility of 170 CRAB isolates, obtained from July to October 2018 at Nopparatrajathanee Hospital, was tested against seven antimicrobial agents by the disk diffusion method. While the susceptibility to colistin was determined by the broth microdilution method. The distribution of carbapenem-resistant genes of *bla*$_{OXA\text{-}51\text{-}like}$, *bla*$_{OXA\text{-}23\text{-}like}$, *bla*$_{OXA\text{-}58\text{-}like}$, and *bla*$_{OXA\text{-}24\text{-}like}$ in the CRAB isolates was determined using a multiplex polymerase chain reaction. The MBL-type carbapenemase genes and IS*Aba1-bla*$_{OXA\text{-}51\text{-}like}$ gene in the CRAB isolates were also detected using conventional PCR. The majority of CRABs (99.42%) were non-susceptible to more than three antimicrobials categories, and 31.18% of CRABs were extensively drug-resistant. Most CRAB isolates (99.42%) were non-susceptible to more than three categories of antimicrobial agents, and 31.18% of CRAB were extensively drug-resistant. Although colistin and tigecycline were the two most effective antimicrobial agents, the resistance rates were 7.06% and 4.12%, respectively. All isolates had the intrinsic resistance gene of *A. baumannii*, the *bla*$_{OXA\text{-}51\text{-}like}$ gene. The frequencies of *bla*$_{OXA\text{-}51\text{-}like}$ with *bla*$_{OXA\text{-}23\text{-}like;}$ *bla*$_{OXA\text{-}51\text{-}like}$ with *bla*$_{OXA\text{-}24\text{-}like}$; *bla*$_{OXA\text{-}51\text{-}like}$ with *bla*$_{OXA\text{-}23\text{-}like}$ and *bla*$_{OXA\text{-}58\text{-}like}$; and IS*Aba1-bla*$_{OXA\text{-}51\text{-}like}$ were 77.06%, 4.71%, 3.53%, and 14.12%, respectively. None of the isolates were positive for MBL-type carbapenemase genes. This research showed that the dominant carbapenems resistance gene among the CRAB in this hospital was *bla*$_{OXA\text{-}23\text{-}like}$. It also confirmed the horror of drug resistance problems for *A. baumannii* with limited treatment options, which should raise everyone's awareness.

Keywords: *carbapenem-resistant Acinetobacter baumannii*; *extensively drug-resistant*; *ISAba1-bla*$_{OXA\text{-}51\text{-}like}$ *gene; OXA-type carbapenemase genes*

1. Introduction

The proliferation of antimicrobial-resistant bacteria is a global health concern, resulting in a significant decrease in the efficacy of drugs for treating bacterial infections. Acinetobacter baumannii is one of the most common multidrug-resistant (MDR) bacteria. The MDR-A. baumannii is a leading cause of nosocomial infection in immunocompromised patients. The ability of A. baumannii to form biofilms makes it resistant to most disinfectants and allows it to survive and adhere to various surfaces in hospital environments, especially medical devices. The prolonged contamination of the hospital environment and medical devices is the source of A. baumannii outbreaks (Hrenovic, Durn, Goic-Barisic, & Kovacic, 2014). MDR-A. baumannii often causes respiratory

infections, especially ventilator-associated pneumonia, which has a mortality rate of 8-35% (Cornejo-Juárez et al., 2020). Carbapenem, a highly effective β-lactam drug, is regularly used for severe bacterial infection treatment, including A. baumannii infections. Excessive use of carbapenems in the treatment of cephalosporin-resistant Gram-negative bacteria is one of the factors that has caused A. baumannii to become carbapenem-resistant A. baumannii (CRAB) (Doi, 2019). The National Antimicrobial Resistance Surveillance, Thailand (NARST) reported that the rate of CRAB infections increased from 14.4% in 2000 to 71.7% in 2016 (NARST, 2018). Several mechanisms are responsible for carbapenem-resistance, but the most common is the production of carbapenemase enzymes that destroy the structure of the drug. Carbapenemase also destroys other β-lactam drugs such as penicillin and cephalosporin (Queenan & Bush, 2007). A. baumannii produces the group of intrinsic oxacillinase enzymes (OXA-51/69) encoded by a $bla_{OXA\text{-}51\text{-}like}$ gene located on the chromosome of A. baumannii, but the expression of the $bla_{OXA\text{-}51\text{-}like}$ gene is low, resulting in only a few effects on the susceptibility to carbapenems. The insertion sequence ISAba1 is upstream of the $bla_{OXA\text{-}51\text{-}like}$ gene, providing the promoter sequences and enhancing the expression of the $bla_{OXA\text{-}51\text{-}like}$ gene, causing a large production of enzyme enough to destroy carbapenems (Evans & Amyes, 2014). The number of enzymes in the OXA-51-like group is up to 95 enzymes (Lob, Hoban, Sahm, & Badal, 2016).

To acquired carbapenem-resistance A. baumannii, the primary mechanisms are enzymes inactivating carbapenems or carbapenemase. These enzymes include the class B metallo-β-lactamases enzyme (MBLs) and carbapenem-hydrolyzing oxacillinases (CHDLs). The MBLs are the β-lactamase enzymes containing zinc ions as an active site for digestion of the β-lactam ring of β-lactam drugs. Thus, metal chelators such as EDTA can inactivate the action of MBL enzymes. The MBLs have been identified in many carbapenem-resistant Gram-negative bacilli but only rarely in A. baumannii. The main types of MBLs in A. baumannii are IMP-like and VIM-like. The encoded genes for IMP-like and VIM-like enzymes are located in the transposons with other resistance genes and can insert into the bacterial chromosomes or within the plasmids (Palzkill, 2013; Poirel & Nordmann, 2006).

The oxacillinases refer to β-lactamases that hydrolyze the oxacillin faster than classical penicillin. The active site of oxacillinases is serine, so EDTA does not affect hydrolytic activity. The subgroups of oxacillinases that can hydrolyze carbapenems are carbapenem-hydrolyzing class D β-lactamases (CHDLs). The CHDLs are also called OXA-type carbapenemase. The efficiency against carbapenems of OXA-type carbapenemases is 100- to 1000-fold lower than MBLs. The OXA-type carbapenemases are frequently reported worldwide in A. baumannii. There are three main groups of acquired OXA-type carbapenemases in A. baumannii, including OXA-23-like, OXA-24-like, and OXA-58-like. The number of enzymes in OXA-23-like, OXA-24-like, and OXA-58-like are 19, 7, and 4 enzymes, respectively. The $bla_{OXA\text{-}23\text{-}like}$ gene was found in at least five transposons, the mobile elements Tn2006 – Tn2009, with the insertion sequences that provide a strong promoter for high expression of the $bla_{OXA\text{-}23\text{-}like}$ gene. The common insertion sequences associated with the $bla_{OXA\text{-}23\text{-}like}$ gene are ISAba1 and ISAba4. The $bla_{OXA\text{-}58\text{-}like}$ gene was found in transposons, with the insertion sequences ISAba3 and ISAba2. The $bla_{OXA\text{-}24\text{-}like}$ gene or $bla_{OXA\text{-}40\text{-}like}$ gene are located on the plasmid (Evans & Amyes, 2014; Nigro & Hall, 2016; Poirel et al., 2006; Poirel & Nordmann, 2006; Walther-Rasmussen & Høiby, 2006). Previous studies reported that most CRAB isolated from the hospitals in Thailand contained $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, and $bla_{OXA\text{-}24\text{-}like}$ genes with different frequencies. (Juntanawiwa et al., 2016; Thirapanmethee et al., 2020).

In the year 2017, Nopparatrajathanee Hospital reported that the percentage of CRAB isolated from clinical specimens was as high as 68%, but the resistance mechanism among CRAB isolates in Nopparatrajathanee Hospital has not been studied.

2. Objectives

1. To determine the prevalence of OXA-type and MBL carbapenemase genes in CRAB isolates from clinical specimens from Nopparatrajathanee Hospital from July to October 2018.
2. To determine the prevalence of multidrug resistance among the CRAB isolates.

3. Materials and methods

3.1 Bacterial isolates

One hundred and seventy CRAB isolates from Nopparatrajathanee Hospital obtained from July-October 2018 were isolated from clinical specimens of non-duplicate patients. The identities of all isolates were confirmed by biochemical tests and rechecked

for carbapenem resistance by disk diffusion testing to imipenem and meropenem disks (Oxoid Limited/UK). Most organisms were isolated from sputum (85.3%). The other specimens were pus 11.2%, urine 2.4%, and blood 1.2%. The positive control strains for OXA-type genes that underwent nucleotide sequence assay and the sequencing of the positive control strains amplicon revealed more than 99% identity with the *A. baumannii* OXA-type genes listed in the GenBank database, including *A. baumannii* AB029 with $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$ genes; *A. baumannii* A300 with $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$, and *A. baumannii* AB126 with IS*Aba1*-$bla_{\text{OXA-51-like}}$ genes. The positive control strains for MBLs genes were *Pseudomonas aeruginosa* AY553332 with $bla_{\text{IMP-14a}}$ gene, *P. aeruginosa* P11 with $bla_{\text{VIM-2}}$, and *Klebsiella pneumoniae* Kp-Ia with $bla_{\text{VIM-1}}$ gene. Ethical approval for this study was obtained from Rangsit University Ethics Review Board (RSUERB2019-048).

3.2 Susceptibility testing

The susceptibility of CRAB against six antimicrobial categories frequently used as *A. baumannii* treatment was performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018). The six antimicrobial categories included β-lactam/β-lactamase inhibitor (piperacillin-tazobactam), extended-spectrum cephalosporin (cefepime), aminoglycoside (gentamicin and amikacin), fluoroquinolone (ciprofloxacin and levofloxacin), glycylcycline (tigecycline) and polymyxin (colistin). The susceptibility of all drugs was determined by the disk diffusion method, with the exception of colistin. The susceptibility of colistin was performed by broth microdilution using the ComASP™ Colistin ranging from 0.25-16 µg/mL (Liofilchem, Inc./Italy).

3.3 Carbapenemase genes detection

The genomic DNA of the CRAB isolates was extracted by boiling at 95°C for 15 minutes, then centrifugation at 12,000 rpm for 15 minutes, according to Alexopoulou et al. (2006). For the detection of the OXA-type genes encoding carbapenemase enzymes, a multiplex-PCR assay was performed according to Woodford et al. (2006). The samples of CRAB that were positive for $bla_{\text{OXA-51-like}}$ gene only were further tested for the upstream IS*Aba1* of $bla_{\text{OXA-51-like}}$ by conventional PCR according to Valenzuela et al. (2007). The detection of MBL genes, $bla_{\text{IMP-like}}$, $bla_{\text{VIM-1-like}}$, and $bla_{\text{VIM-2-like}}$ in the CRAB samples was performed by conventional PCR according to Lee et al. (2005). The primer pairs used for the detection of carbapenemase-encoding genes are listed in Table 1. All PCR assays were performed in a thermal cycler (TC-3000, Barloworld Scientific Ltd./UK).

Table 1 The primers sequences for specific amplification of OXA-carbapenemase genes, MBL-carbapenemase genes, and IS*Aba1*-$bla_{\text{OXA-51-like}}$ gene of the CRAB isolates

Gene	Primer Name	Primer Sequence (5' to 3')	Product Size	Reference
$bla_{\text{OXA-23-like}}$	OXA23-F	GATCGGATTGGAGAACCAGA	501 bp	Woodford et al., 2006
	OXA23-R	ATTTCTGACCGCATTTCCAT		
$bla_{\text{OXA-24-like}}$	OXA24-F	GGTTAGTTGGCCCCCTTAAA	249 bp	Woodford et al., 2006
	OXA24-R	AGTTGAGCCAAAAGGGGATT		
$bla_{\text{OXA-51-like}}$	OXA51-F	TAATGCTTTGATCGGCCTTG	353 bp	Woodford et al., 2006
	OXA51-R	TGGATTGCACTTCATCTTGG		
$bla_{\text{OXA-58-like}}$	OXA58-F	AAGTATTGGGGCTTGTGCTG	599 bp	Woodford et al., 2006
	OXA58-R	CCCCTCTGCGCTCTACATAC		
IS*Aba1*-$bla_{\text{OXA-51-like}}$	ISAba1 F	TCTAACGACGAATACTATGAC	371 bp	Valenzuela et al., 2007
	OXA51 R	TGGATTGCACTTCATCTTGG		
$bla_{\text{IMP-like}}$	IMP-F	CATGGTTTGGTGGTTCTTGT	488 bp	Lee et al., 2005
	IMP-R	ATAATTTGGCGGACTTTGGC		
$bla_{\text{VIM 1-like}}$	VIM 1-F	ATGTTAAAAGTTATTAGTAGT	801 bp	Lee et al., 2005
	VIM 1-R	CTACTCGGCGACTGAGCGAT		
$bla_{\text{VIM 2-like}}$	VIM 2-F	ATGTTCAAACTTTTGAGTAAG	801 bp	Lee et al., 2005
	VIM 2-R	CTACTCAAC GACTGAGCGAT		

3.4 Carbapenemase enzyme assays

The carbapenemase production in the CRAB isolates was tested by the modified carbapenem inactivation method (mCIM). When combined with the EDTA-CIM(eCIM) the assay can distinguish the types of carbapenemase. The mCIM/eCIM assay was performed following the guidelines of CLSI (2018). In the mCIM tube, a 10-

μL loopful of tested CRAB colonies was inoculated into 2 mL of tryptic soy broth (TSB, Becton, Dickinson, and Co./France). In the eCIM tube, a 10-μL loopful of tested CRAB colonies was inoculated into 2 mL of TSB with 20 μL of 0.5 M EDTA (Thermo Scientific /USA). Then a 10 μg meropenem disk was added (Oxoid Limited/UK) to both tubes, followed by incubation at 35°C. After 4 hours, the disks were removed from the mCIM and eCIM tubes, and applied to Mueller-Hinton agar plates that were freshly inoculated with 0.5 McFarland suspension of *Escherichia coli* ATCC 25922, a carbapenem-susceptible strain. The plates were incubated at 35°C for 16 to 20 hours. The tested strain was interpreted as positive for the production of carbapenemase when the zone diameter of the meropenem disk from the mCIM tube was 6-15 mm or 16-18 mm with small colonies in the inhibitory zone. The eCIM result was only read when the mCIM was positive. The tested strain was interpreted as a metallo-carbapenemase producer when the eCIM zone diameter was 5 mm greater than the mCIM zone diameter. If not, the tested strain was interpreted as a non-metallo carbapenemase producer or a serine carbapenemase producer (CLSI, 2018).

4. Results and discussion

The carbapenem-resistance in *A. baumannii* isolates from clinical specimens from Nopparatrajathanee Hospital increased from 68% in 2017 to 77.69% in 2018. All 170 CRAB samples were resistant to both imipenem and meropenem. The susceptibility testing of the CRAB against other drugs for *A. baumannii* treatment showed high resistance rates. All isolates were resistant to piperacillin/tazobactam, and the resistance rate of cefepime was 99.41%. The CRAB was resistant to most β-lactam because carbapenem-hydrolyzing oxacillinases can hydrolyze almost all β-lactam drugs and resistant to most β-lactamase inhibitors. The resistance rate of CRAB isolates to fluoroquinolones and aminoglycosides was 81.76 - 99.41% and 9.41- 35.88%, respectively. The resistance rate of colistin and tigecycline, the two most effective treatments for multidrug-resistant Gram-negative bacteria, was 7.06% and 4.12%, respectively.

The susceptibility test results of 170 CRAB isolates showed 20 different antimicrobial susceptibility patterns (antibiograms). The number of CRAB isolates in each antibiogram ranged from 1-71 isolates. Most CRAB isolates (99.41%) were non-susceptible to more than two antimicrobial categories. Moreover, 38.24% of the CRAB isolates were extensively drug-resistant (XDR), which are non-susceptible to all but one or two antimicrobial categories. Fortunately, the pandrug-resistant strain of *A. baumannii* was not detected (Magiorakos et al., 2012). The susceptibility results of CRAB isolates are shown in Table 2. All CRAB isolates were mCIM positive, indicating that all isolates produced carbapenemase. However, the results of the MBL-enzyme detection of CRAB isolates by eCIM were negative, showing that all CRAB samples in this study produced serine carbapenemase enzyme.

All isolates exhibited the *bla*$_{\text{OXA-51-like}}$ gene, an intrinsic resistance gene of *A. baumannii*, which confirmed the biochemical tests that the isolates in this study were *A. baumannii* (Turton et al., 2006). The detection of acquired resistance of *bla*$_{\text{OXA}}$ genes by multiplex PCR revealed that 81.18% of isolates were positive for the *bla*$_{\text{OXA-23-like}}$ gene. An isolate that was susceptible to cefepime was isolated from sputum and harbored *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{OXA-51-like}}$ genes. The genetic information of this isolate, such as the insertion sequences may be needed, as well as other carbapenem-resistance mechanisms, such as the loss of outer-membrane proteins (25/29-kDa OMP or 22/33 kDa OMP) or the alteration of penicillin-binding protein, may also be needed (Bou, Cerveró, Domínguez, Quereda, & Martínez-Beltrán, 2000; Vashist, Tiwari, Das, Kapil, & Rajeswari, 2011). In contrast, the prevalence rates of the *bla*$_{\text{OXA-24-like}}$ and *bla*$_{\text{OXA-58-like}}$ genes in CRAB isolates were only 4.71% and 3.53%, respectively. Other reports of prevalence rates of the *bla*$_{\text{OXA}}$ genes of *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-24-like}}$, and *bla*$_{\text{OXA-58-like}}$ genes in CRAB isolates in Thailand ranged from 68.31-97.9%, 0-4.92%, and 1.09-6.5%, respectively (Juntanawiwat, Thunyaharn, Visawapoka, Samosornsuk, & Samosornsuk, 2016; Leungtongkam et al., 2018; Thirapanmethee et al., 2020). In this study, 24 isolates harbored only the *bla*$_{\text{OXA-51-like}}$ gene, but the mCIM/eCIM results indicated that they produced serine-carbapenemase. Therefore, further conventional PCR was performed to detect the insertion sequence IS*Aba1* upstream of the *bla*$_{\text{OXA-51-like}}$ gene and found that all 24 isolates were positive for the IS*Aba1*-*bla*$_{\text{OXA-51-like}}$ gene. The insertion sequence IS*Aba1* at the upstream position of the *bla*$_{\text{OXA-51-like}}$ gene can enhance the expression

of *bla*$_{\text{OXA-51-like}}$ gene, causing *A. baumannii* to produce a large amount of oxacillinase enough to destroy carbapenems (Evans & Amyes, 2014). The prevalence of OXA-type carbapenemase genes in CRAB isolates from Nopparatrajathanee Hospital is shown in Table 3. The PCR product patterns of four groups of *bla*$_{\text{OXA}}$ genes are shown in Figure 1. There were no significant differences in antibiograms among each OXA-type gene, but we did not compare the minimum inhibitory concentration of imipenem and meropenem among OXA-type genes. The molecular detection of the MBLs genes showed that none of the isolates had the MBL-encoding genes, which corresponded to the mCIM/eCIM that all CRAB produced non-metallo-carbapenemase.

The outbreak of CRABs in Nopparatrajathanee Hospital from July to October 2018 was due to the production of OXA-type carbapenemase. The majority of OXA-type carbapenemase in the CRAB was OXA-23-like enzymes. In this study, most CRABs were isolated from the sputum of patients with ventilator-associated pneumonia. The ability to form biofilms of *A. baumannii* allowed it to adhere to the ventilator tube and become the main source of *A. baumannii* outbreaks. Therefore, most *A. baumannii* infections were nosocomial infections. The source of nosocomial infections may be the endemic *A. baumannii* clones in the hospital environment. Other sources of CRAB infection are the endogenous source of *A. baumannii* colonized in the respiratory tract and digestive systems of the patients and healthcare personnel. Therefore, some *A. baumannii* infections may be caused by the different *A. baumannii* clones. Although the community-acquired infection (CAI) of *A. baumannii* is rare, the severity of CAI has been reported to increase in patients with underlying diseases. There was no report on the length of time for each patient's hospitalization before having an infection. Therefore, information on nosocomial and community-acquired *A. baumannii* infection of each isolate was lacking. To confirm that there were the clones of *A. baumannii* that harbored *bla*$_{\text{OXA-23-like}}$ in the hospital environment would require further genotypic studies of the CRAB isolates form clinical specimens, environment and medical devices, according to the report of Chen et al., (2018) and Nigro and Hall (2016).

Table 2. Results of antimicrobial susceptibility testing of carbapenem-resistant *A. baumannii* isolates (n = 170)

Antimicrobial category	Antimicrobial agent	Susceptible		Intermediate		Resistant	
		N	%	N	%	N	%
Antipseudomonal penicillins + β-lactamase inhibitors	Piperacillin/tazobactam	0	0	0	0	170	100
Extended-spectrum cephalosporin	Cefepime	1	0.59	0	0	169	99.41
Fluoroquinolones	Ciprofloxacin	1	0.59	0	0	169	99.41
	Levofloxacin	5	2.94	26	15.29	139	81.76
Aminoglycosides	Gentamicin	83	48.82	26	15.29	61	35.88
	Amikacin	136	80	18	10.59	16	9.41
Polymyxins	Colistin	158	92.94	0	0	12	7.06
Glycylcycline	Tigecycline	113	66.47	50	29.41	7	4.12

Table 3. The prevalence of *bla*$_{\text{OXA}}$ genes in carbapenem-resistant *A. baumannii* isolates

OXA-type	Carbapenem-resistant *A. baumannii* (n = 170)	
	N	%
bla$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-23-like}}$	131	77.06
bla$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-24-like}}$	9	5.29
bla$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-58-like}}$	6	3.53
IS*Aba1*-*bla*$_{\text{OXA-51-like}}$	24	14.12

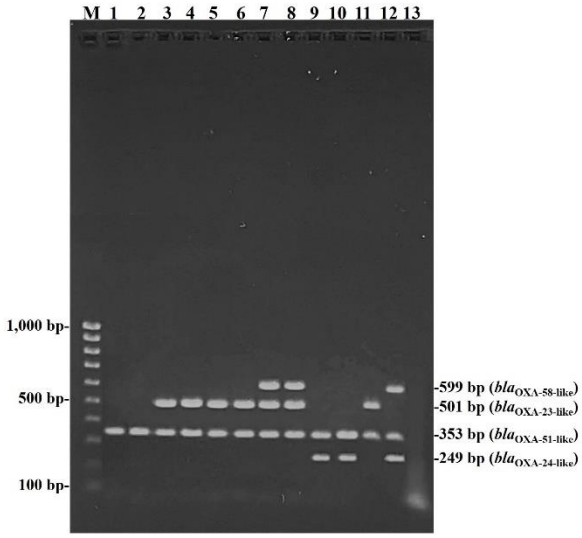

Figure 1 Detection of OXA-type carbapenemase genes by multiplex PCR, lane M, 100 bp DNA marker (HyperLadder^{TM}); 1-2, *bla*$_{OXA-51-like}$ (AB050, AB116); 3-6, *bla*$_{OXA-51-like}$ and *bla*$_{OXA-23-like}$ (AB008, AB016, AB035, AB071); 7-8, *bla*$_{OXA-51-like}$, *bla*$_{OXA-23-like}$ and *bla*$_{OXA-58-like}$ (AB054, AB142), 9-10, *bla*$_{OXA-24-like}$ and *bla*$_{OXA-51-like}$ (AB182, AB183), lane 11, positive control for *bla*$_{OXA-51-like}$ and *bla*$_{OXA-23-like}$ (AB029), lane 12, positive control for *bla*$_{OXA-24-like}$, *bla*$_{OXA-51-like}$ and *bla*$_{OXA-58-like}$ (A300); and lane 13, negative control, distilled water.

5. Conclusion

This study found that most CRAB isolates (99.41%) were multidrug-resistant, with a high resistance rate to β-lactam drugs and fluoroquinolones, and in this group, 31.18% were XDR-*A. baumannii*, suggesting that many CRAB strains may harbor many other resistance genes, increasing the risk of death in patients with CRAB infection, as the options of the drugs for the CRAB treatment are very rare. The detection of carbapenemase enzyme production by mCIM/eCIM method as well as MBL-carbapenemases genes detected by PCR indicated that none of the CRAB isolates produced MBL-carbapenemase (Lee et al., 2003; Walsh, Toleman, Poirel, & Nordmann, 2005). Among carbapenem resistance mechanisms of *A. baumannii*, our studies suggested that the production of OXA-type carbapenemases, especially the OXA-23-like enzyme, was most commonly found in *A. baumannii* isolated from clinical specimens in Nopparatrajathanee Hospital. Our results were consistent with those of previous studies which described the spread of OXA-23-producing *A. baumannii* strains in various locations worldwide, with a high prevalence in Thailand (Thirapanmethee et al., 2020; Wang, Leu, Wang, Liu, & Yan, 2018). This study detected small percentages of the *bla*$_{OXA-24-like}$, and *bla*$_{OXA-58-like}$ genes in the CRAB isolates.

Our study confirms that we are facing a critical problem of multidrug-resistant *A. baumannii* with limited therapeutic options. Early detection of carbapenem-resistant *A. baumannii* by a rapid, specific, sensitive, and convenient method can control the spread of CRAB infection. The molecular biology assays for antimicrobial resistance bacteria detection, especially carbapenem-resistant *A. baumannii*, maybe the key for controlling the resistance problems.

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