Detection of Bla_{IMP} and $Bla_{OXA-23-LIKE}$ Genes in *Acinetobacter Baumannii* Isolates at Dr. Wahidin Sudirohusodo Hospital

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Abstract

Background/Objective: Carbapenem resistant *Acinetobacter baumannii* (CRAB) is an *A. baumannii* resistant to one or more carbapenem antimicrobials. Some of resistance mechanisms that can be found in CRAB are carbapenemase production, outer membran protein (OMP) or penicillin binding proteins (PBP) structure modification, or efflux pump increase. Carbapenemase production can be affected by carbapenemase-encoding genes such as bla_{IMP} and $bla_{OXA23\text{-}like}$ gene. This study aimed to detect the bla_{IMP} and $bla_{OXA23\text{-}like}$ gene in *A. baumannii* isolates.

Material and Method : It was observational descriptive study with cross sectional approach. Subject of this study were all *A. baumannii* isolates collected during the period of October 2018 until January 2019 at Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, Indonesia (consisted of 30 CRAB and 20 sensitive carbapenem *A. baumannii* (CSAB) isolates). Isolates were examined by polymerase chain reaction (PCR) and agar electrophoresis techniques.

Results : There were 5 CRAB isolates with bla_{IMP} gene and 23 isolates with $bla_{OXA23\text{-}like}$ gene. There were no CSAB isolates with bla_{IMP} gene and 2 isolates with $bla_{OXA23\text{-}like}$ gene.

Conclusion : Carbapenemase-encoding genes could be found in CRABs and CSABs. In this study, $bla_{OXA23\text{-}like}$ gene was found in both CSAB and CRAB isolates but bla_{IMP} gene was only found in CRAB isolates.

Keywords: *Acinetobacter baumannii*, *carbapenem resistant*, *carbapenem sensitive*, *bla_{IMP}*, *$bla_{OXA\text{-}23\text{-}like}$*

Introduction

Antimicrobial resistance (AMR) is the ability of microorganisms to survive from one or more antimicrobials which are initially effective for treating infections caused by these microorganism. The development of AMR is a natural phenomenon caused by bacterial genes mutation, or the acquisition of extrinsic resistance genes that can be transmitted horizontally between bacteria. Bacteria can have different resistance mechanism simultaneously so that they become resistant to several classes of antibiotics.[1,2]

Acinetobacter baumannii is one of extended-spectrum β-lactamases (ESBLs) bacteria group. Carbapenem resistant *A. baumannii* is *A. baumannii* that is resistant to one or more carbapenems (imipenem, meropenem, atau doripenem). Carbapenems, among all the **β-lactam antibiotics**, are able to withstand many types of β-lactamases produced by the ESBLs bacteria so they become drug of choice for ESBLs. Excessive use of carbapenems in last few years has caused the emergence of CRAB.[3–5]

The main mechanism of carbapenem resistance is carbapenem inactivation by carbapenemases, especially oxacillinases (OXAs) and metallo-β-lactamases (MBLs) type. Carbapenemases such as OXA-58, OXA-24/40, OXA-23, and OXA-51 (OXAs) or imipenem hydrolyzing β-lactamase (IMP) and verona integron-encoded metallo-β-lactamase (VIM) (MBLs) often found in *A.baumannii*. These enzymes are produced by carbapenemase-encoding genes such as $bla_{OXA\text{-}58}$, $bla_{OXA\text{-}24/40}$, $bla_{OXA23\text{-}like}$, $bla_{OXA\text{-}51}$, bla_{IMP}, or bla_{VIM} respectively. The other mechanism is related to the smaller number and size of OMP compared to other gram-negative bacteria thus reducing the permeability

of bacteria to antibiotics. Other mechanisms that can be found in CRAB are active expulsion of antibiotics as soon as they enter bacterial membrane wall through the efflux pump system and modification of PBP thus decreasing the affinity of bacteria for antibiotics.[3,4,6–9]

This study aimed to detect the bla_{IMP} and $bla_{OXA23\text{-}like}$ gene in *A. baumannii* isolates in Dr. Wahidin Sudirohusodo Hospital, Makassar.

Materials and Method

Design and Subject

The study was observational descriptive with cross sectional approach. Subject of this study were all *A. baumannii* isolates during the period of October 2018 until January 2019 at the Central Laboratory of Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, Indonesia that met the inclusion criteria.

The inclusion criteria was *A. baumannii* isolat identified by Vitek® 2 Compact and had antibiotics sensitivity test result based on Clinical and Laboratory Standards Institute (CLSI) guidelines. The *A. baumannii* isolate was excluded if isolat was contaminated by other bacteria. Isolates were examined by PCR and agar electrophoresis techniques to detect the bla_{IMP} and $bla_{OXA23\text{-}like}$ genes.

Acinetobacter baumannii is coccobacillus bacteria isolat identified by Vitek® 2 Compact. The CSAB is *A. baumannii* which is sensitive to all carbapenems (imipenem meropenem, ertapenem, dan doripenem) identified by antibiotics sensitivity test with MIC ≤ 2. The CRAB is *A. baumannii* which is resistant to one or more carbapenems (imipenem meropenem, ertapenem, dan doripenem) identified by antibiotics sensitivity test with MIC ≥ 8. The bla_{IMP} gene is MBLs type carbapenemase-encoding gene identified if a band is found at position 183 bp by PCR and electrophoresis agar techniques. The $bla_{OXA\text{-}23\text{-}like}$ is OXAs type carbapenemase-encoding gene identified if a band is found at position 736 bp by PCR and electrophoresis agar techniques.

Ethic

Ethical clearence had been accepted before study from Medical Research Ethics Committee of Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia.

Statistic Analysis

The data was analyzed using descriptive statistic method.

Result and Discussion

55 *A. baumannii* isolates consisted of 30 CRAB and 20 CSAB isolates were collected during this research. *Acinetobacter baumannii* isolates were more commonly found in male patients (52.7%). This fact is in line with research by Gustawan et al. (2014), An et al. (2017), and Irfan et al. (2011) who also found that *A. baumannii* isolates were more common in males patients.[10-14]

Acinetobacter baumannii isolates were more commonly found in group of age 40-60 years (38.2%). Research by An et al. (2017) reported that the average age of patients with CSABs was 64 ± 15 and 63 ± 15 for patients with CRABs. Research by Zheng et al. (2013) also reported that the mean age of patients with CSABs was 60.9 ± 9.8 and 62.2 ± 9.7 for patients with CRABs. This shows that *A. baumannii* infection is often affecting people with old age. This is probably because older patients usually have other comorbid factors and also experience a decline in the immune system so they are susceptible to *A. baumannii* infection.[10,14-15]

The majority of *A. baumannii* isolates in this research came from sputum (54.6%). This fact is in line with the research of Cucunawangsih et al (2016), Sarmad and Eftekhar (2015), and Chang et al. (2015) who reported that most of *A. baumannii* isolate was obtained from sputum. *Acinetobacter spp.* generally considered part of the normal flora of the pharyngeal mucous membranes and secretions of human respiration so that these bacteria are often found from respiratory secretions such as sputum.[7,12,16-18]

Acinetobacter baumannii isolates were found most frequently in patients with infectious diseases (56.4%) especially pneumonia and sepsis. This is in line with the research of Kulah et al. (2010) and An et al. (2017). Tal-Jasper et al. research on bloodstream infections by *A. baumannii* in 2016 found that these infections were mostly derived from previous pneumonia. *Acinetobacter baumannii* is often involved in various diseases such as pneumonia, osteomyelitis, peritonitis, endocarditis, septicemia and meningitis. Several studies had shown that patients with burns were also susceptible to *A. baumannii* infections during hospitalization.[10,19-21]

The majority of patient outcomes, as many as 61.8%, were discharged from the hospital in improved condition while the rest died in the hospital. Most of the patients who died in the hospital (13 person) were CRABs positive. Only 8 patients with CSABs positive. Tal-Jasper et al. research (2016) found out that the percentage of patients with CRABs died in the hospital was greater than CSABs (70.5% compared to 40.5%). Gustawan et al. (2014) found that most of patient outcomes in *A. baumannii* infections were died in the hospital.[11,21]

The average length of stay (LOS) patients with CRABs was 40 days. This was longer than average LOS patients with CSABs (13.9 days). This is in line with the research of Tal-Jasper et al. (2016) who found LOS patients with CRABs was longer than CSABs. The characteristics of this research samples can be seen in Table 1.[11,21]

Table 1. Characteristics of Research Samples

Research Samples Characteristics		n (%)	CSAB	CRAB
Sex	Male	29 (52,7)	11	18
	Female	26 (47,3)	14	12
Age	< 20 years	13 (23,6)	3	10
	20-40 years	15 (27,3)	6	9
	40-60 years	21 (38,2)	11	10
	> 60 years	6 (10,9)	5	1
Specimens	Pus	13 (23,6)	1	12
	Sputum	30 (54,6)	15	15
	Blood	5 (9,1)	5	0
	Urine	5 (9,1)	3	2
	Pleural fluid	1 (1,8)	1	0
	Faeces	1 (1,8)	0	1
Diagnosa	Infection disease	31 (56,4)	15	16
	Injury	10 (18,2)	1	9
	Malignancy	7 (12,7)	4	3
	Degenerative disease	7 (12,7)	5	2
Patient Outcomes	Improved	34 (61,8)	17	17
	Died	21 (38,2)	8	13
Median Hospital Length of Stay			40,0 (6-233)	13,9 (0-37)
CSAB - carbapenem sensitive A. baumannii, CRAB - carbapenem sensitive A. baumannii				

Sources : Primary Data

Most of the CRABs were found in intensive care rooms such as Neonatus Intensive Care Unit (NICU), Pediatric Intensive Care Unit (PICU), dan Intensive Care Unit (ICU). The CRABs were most commonly found in the ICU. The CRABs were also found in regular hospital rooms with the most isolates found in Lontara 2 rooms. Only 1 CSAB was found in the ICU and 2 in the PICU. Most of CSABs were found in the Lontara 1 rooms. *Acinetobacter baumannii* emerges as an important pathogen in critical care settings in the recent years. Outbreaks caused by A. baumannii were often found in ICUs and burns injury care units. The distribution of CSAB and CRAB based on the treatment room can be seen in Figure 1.[16,19-20]

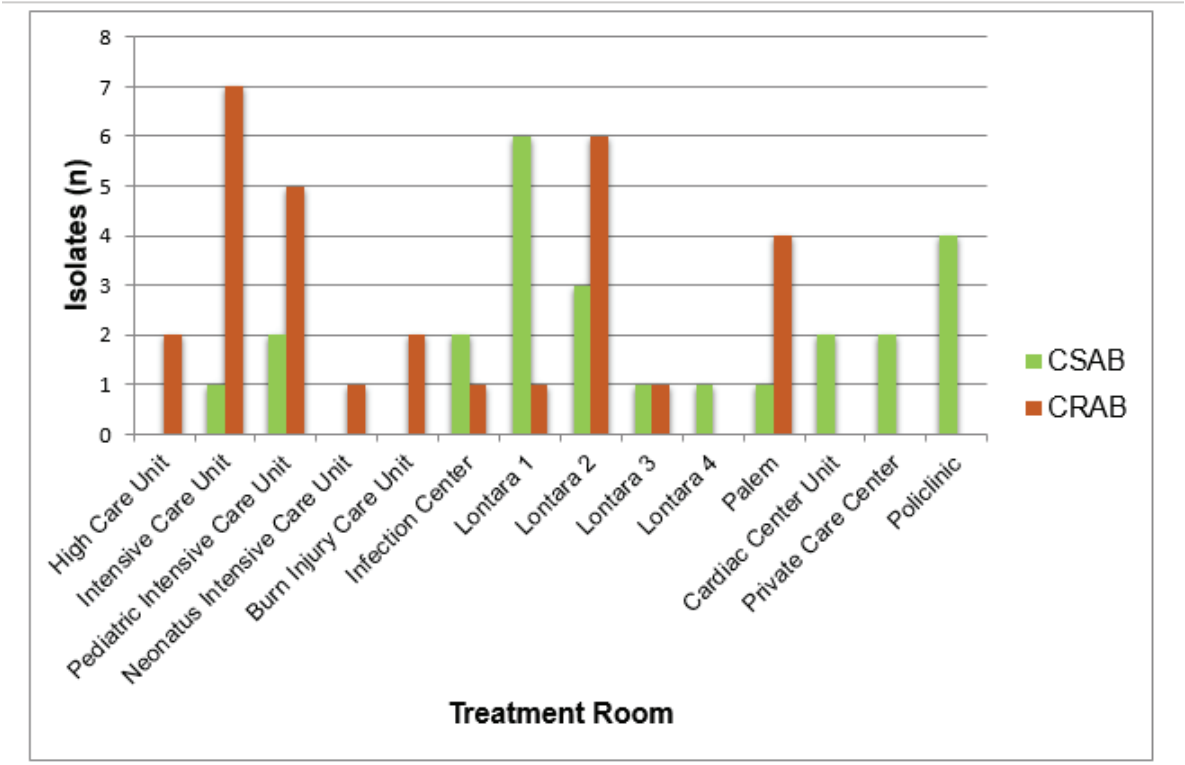

Figure 1. *Carbapenem Sensitive A. baumannii* **(CSAB) and** *Carbapenem Resistant A. baumannii* **(CRAB) Distribution Based on Treatment Room**

There were 5 CRABs with bla_{IMP} gene and 23 with $bla_{OXA23\text{-}like}$ gene. There were no CSAB with bla_{IMP} gene and 2 with $bla_{OXA23\text{-}like}$ gene. The distribution bla_{IMP} and $bla_{OXA\text{-}23\text{-}like}$ genes can be seen in Table 2.

Table 2. Bla_{IMP} and $Bla_{OXA\text{-}23\text{-}like}$ Genes Distribution

Gen	CRAB		CSAB	
	Positif	Negatif	Positif	Negatif
IMP	5	25	0	25
blaOXA-23-like	23	7	2	23

The results of this study indicates bla_{IMP} and $bla_{OXA\text{-}23\text{-}like}$ genes as carrier genes for the carbapenem resistance characteristics of *A. baumannii*. These results are in line with Santimaleeworagun et al. (2014) research who found 42 out of 43 CRABs had $bla_{OXA\text{-}23}$ gene. Amiri et al. research (2017) found 85.2% from 27 CRABs had the $bla_{OXA\text{-}23\text{-}like}$ genes ($bla_{OXA\text{-}23}$, $bla_{OXA\text{-}27}$, $bla_{OXA\text{-}49}$).[22-24]

This study reported that carbapenemase-encoding gene also found in the CSABs, especially $bla_{OXA-23-like}$ gene. This is likely to occur because these genes can be transmitted to other bacteria through acquired resistance mechanisms such as the acquisition of mobile genetic elements that are able to spread the determinants of resistance. There are 3 classic methods of natural deoxyribonucleic acid (DNA) transfer, namely bacterial conjugation, natural transformation, and transduction. Conjugation is often regarded as the main transfer mechanism. Exogenous DNA can be transferred from one bacterium to another through horizontal gene transfer. The discovery of $bla_{OXA-23-like}$ gene in CSABs shows some CSABs already have a tendency to have carbapenem resistant genotype even though they have not been maximally expressed so that the results of antibiotic sensitivity test are still sensitive.[25-26]

Negative results on CRABs in this research may be caused by the presence of other carbapenemase-encoding genes outside the genes examined in this research such as the intrinsic gene bla_{AmpC} and bla_{OXA-51} or the extrinsic gene $bla_{OXA-24/40}$, bla_{OXA-58}, bla_{VIM}, or bla_{SIM}. Another possibility that can occur is the existence of other carbapenem resistance mechanisms such as the active expulsion mechanism mediated by the tripartite efflux pump system and PBP or OMP structure modification.[6,9,27-28]

The limitation of this study is that there was no examination of the carbapenemase phenotype in *A. baumannii* isolates using a modified double-disk synergy test (DDST) to confirm that these isolates were true carbapenemase-producing type. This research also was not researching other carbapenem resistance mechanisms.

Conclusion

Carbapenemase-encoding genes could be found in CRABs and CSABs. In this study, $bla_{OXA23-like}$ gene was found in both CSAB and CRAB isolates but bla_{IMP} gene was only found in CRAB isolates.

Conflict of Interest : None

Source of Funding : Self

Ethical Clearence : Obtained from Medical Research Ethics Committee of Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia

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