

Original Article

The susceptibility pattern and distribution of bla_{OXA-23} genes of clinical isolate *Acinetobacter baumannii* in a tertiary hospital, IndonesiaDewi Anggraini^{1,2,3}, Rahmat Azhari Kemal⁴, Usman Hadi^{5,7}, Kuntaman Kuntaman^{6,7}¹ Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia² Department of Microbiology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia³ Arifin Achmad General Hospital, Pekanbaru, Indonesia⁴ Department of Medical Biology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia⁵ Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia⁶ Department of Medical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia⁷ Dr. Soetomo General Academic Hospital, Surabaya, Indonesia**Abstract**

Introduction: *Acinetobacter baumannii*, a pathogen of concern in hospitals worldwide, has diverse antimicrobial resistance mechanisms leading to limiting the antibiotic options and carbapenemase enzyme production is one of the common mechanisms in carbapenem resistance. The epidemiology and resistance pattern of clinical isolates are critical in developing a prevention and treatment strategy. The aim of this was to determine the prevalence and resistance pattern of carbapenem non-susceptible strains (CNS) *A. baumannii* at Arifin Achmad General Hospital, Pekanbaru, Indonesia.

Methodology: Data were retrieved from the culture and susceptibility test results from various clinical specimens from January 2015 to December 2019. A susceptibility test was conducted using Vitek 2 Compact following the manufacturer's protocol. To explore the genetic profile of CNS *A. baumannii*, we amplified the bla_{OXA-23} and bla_{OXA-51} genes, carbapenemase producing genes, using a duplex polymerase chain reaction (PCR) among 24 isolates. Chi-squared was used to assess the factors associated with the presence of CNS *A. baumannii*.

Results: Between 2015-2019, 1,263 *A. baumannii* isolates were tested and the prevalence of CNS *A. baumannii* was 50%. The trend decreased from 53% in 2016 to 45% in 2019. The proportion of CNS *A. baumannii* was higher among samples from patients treated in the Intensive Care Unit (ICU) compared to non-ICU ($p < 0.001$). The CNS *A. baumannii* was also more frequently detected from sputum than from non-sputum samples ($p = 0.009$). CNS *A. baumannii* were highly resistant to almost all antibiotics and the highest susceptibility was to amikacin, tigecycline, and trimethoprim/sulfamethoxazole with 64%, 53%, and 43%, respectively. The bla_{OXA-23} gene was detected in 92% of tested CNS *A. baumannii* isolates.

Conclusions: The prevalence of CNS *A. baumannii* is high at Arifin Achmad Hospital Riau, Indonesia. This is also supported by the high prevalence of the bla_{OXA-23} gene among tested isolates. Based on the antibiotic susceptibility pattern there are limited antibiotic choices for CNS *A. baumannii* urging the strengthening of antimicrobial stewardship programs in the country.

Key words: Infectious disease; *Acinetobacter baumannii*; carbapenemase; Indonesia; antibiotic susceptibility.

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Introduction

Acinetobacter baumannii is a Gram-negative, oxidase-negative, non-fastidious, and strictly aerobic bacteria that has become a health significant problem globally [1]. It can accumulate various antibiotic resistance mechanisms, resulting in resistant strains to most available antibiotics [2]. *A. baumannii* has become one of the primary causes of nosocomial infection in healthcare settings, particularly in the Intensive Care Unit (ICU). In addition, the bacteria are also associated with war- and natural disaster-related infections [3]. *A. baumannii* belongs to the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*,

Klebsiella pneumoniae, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*), a group of six highly virulent and antibiotic-resistant bacterial pathogens and associated with healthcare-related infections with the potential for substantial antimicrobial resistance [4]. Additionally, carbapenem-resistant *A. baumannii* is a critical priority pathogen and is a part of the World Health Organization (WHO) priority list of antibiotic-resistant bacteria for effective drug development [5].

Carbapenem is a last-line β -lactam antibiotic commonly prescribed to patients in critical condition and patients with multiple drug-resistant Gram-

negative bacterial infections [6,7]. The prevalence of carbapenem non-susceptible (CNS) *A. baumannii* is high globally [7]. Carbapenem resistance increases the risk of mortality in a patient with *A. baumannii* infection [8]. The main mechanism of resistance to carbapenem is the production of carbapenemase enzyme. The dominant carbapenemase gene in *A. baumannii* is *bla_{OXA-23}*. There is also *bla_{OXA-51}* in the carbapenemase gene intrinsic in *A. baumannii*; therefore, it is usually used to identify this bacteria [9–13].

In low- and medium-income countries (LMICs) in the South and Southeast Asia region, the prevalence of CNS *A. baumannii* is reportedly high [7]. Studies on multi-drug resistant *A. baumannii* in Indonesia are still limited. This study aimed to determine the prevalence and antibiotic susceptibility pattern of CNS *A. baumannii* in a tertiary teaching hospital, Pekanbaru, Indonesia.

Methodology

Research design

A cross-sectional study was conducted at Arifin Achmad General Hospital. Data for culture examination and susceptibility test results from various clinical specimens between 1st January 2015 and 31st December 2019 were retrieved retrospectively from the Microbiology Department and Clinical Pathology Laboratory of Arifin Achmad General Hospital. Samples with incomplete data such as data without antibiotic susceptibility test results, source of the sample, and type of specimen were excluded. To confirm the CNS *A. baumannii*, a duplex polymerase chain reaction (PCR) was conducted to detect the *bla_{OXA-51}* and *bla_{OXA-23}* genes.

Antibiotic susceptibility test and definition of CNS *A. baumannii*

A. baumannii isolates were a collection from the routine procedure at the Laboratory of Microbiology in this hospital. Specimens were inoculated onto blood agar and MacConkey agar, incubated at 37 °C overnight, and a suspected colony of non-fermenter were selected for further identification. The diagnosis and antimicrobial susceptibility testing was conducted by VITEK 2 Compact automated diagnostic machine (VK2C19207) (Biomérieux, Marcy-l’Etoile, France). Antibiotics tested were amikacin, ceftazidime, ciprofloxacin, ceftriaxone, cefazoline, cefepime, gentamycin, meropenem, ampicillin/sulbactam, trimethoprim/sulfamethoxazole, tigecycline, and piperacillin/tazobactam. CNS *A. baumannii* was

defined as *A. baumannii* which was not sensitive to meropenem, imipenem, or doripenem. However, in this study we performed the susceptibility test for meropenem only due to the limitation of the available AST card of VITEK 2 in the country: AST GN93 only was available. Therefore, CNS *A. baumannii* in this study represents meropenem non-susceptible strain *A. baumannii* only.

Amplification of *bla_{OXA-51}* and *bla_{OXA-23}*

All CNS *A. baumannii* isolates from August 2019 were subjected to a confirmation test. There were 24 CNS *A. baumannii* isolates between 1st and 31st August 2019 and were used for confirmation PCR by detecting *bla_{OXA-51}* and *bla_{OXA-23}* genes. The genomic DNA was extracted with Wizard Genomic DNA Kit (Promega, Madison, USA) according to manufacturer instructions. The PCR amplification of *bla_{OXA-51}* and *bla_{OXA-23}* genes were conducted using a set of primers available from a previous study [14]. The 2X GoTaq Green Master-Mix (Promega, Madison, USA) was used for the PCR amplification. The amplification conditions were: initial denaturation at 95 °C for 2 minutes, followed by 33 cycles of 95 °C for 25 seconds, 57 °C for 40 seconds, and 72 °C for 50 seconds, with the final elongation at 72 °C for 5 minutes. The PCR products were analyzed by electrophoresis in 2% (w/v) agarose gel stained with GelRed Nucleic Acid Gel Stain with BenchTop DNA ladder 100 bp (Promega, Madison, USA). Electrophoresis result was visualized using the GelDoc system (Biorad, Hercules, USA). The sizes of *bla_{OXA-51}* and *bla_{OXA-23}* were 353 base-pairs (bps) and 501 bps, respectively. Positive control was obtained from the Clinical Microbiology Laboratory, Department of Microbiology, Faculty of Medicine, Universitas Indonesia in Jakarta. The positive control isolate has been confirmed to harbor *bla_{OXA-51}* and *bla_{OXA-23}* in Erasmus MC, Netherlands.

Data analysis

The data on antibiotic susceptibility, wards, and specimen type were retrieved from WHONET 5.6 software. Plausible factors associated with the presence of CNS *A. baumannii* such as type of wards, unit and type of sample were assessed using the Chi-squared test.

Ethical consideration

The protocol of this study was approved by the Ethical Committee Medical Faculty, Universitas Riau (#090/UN.19.5.1.1.8/UEPKK/2019).

Results

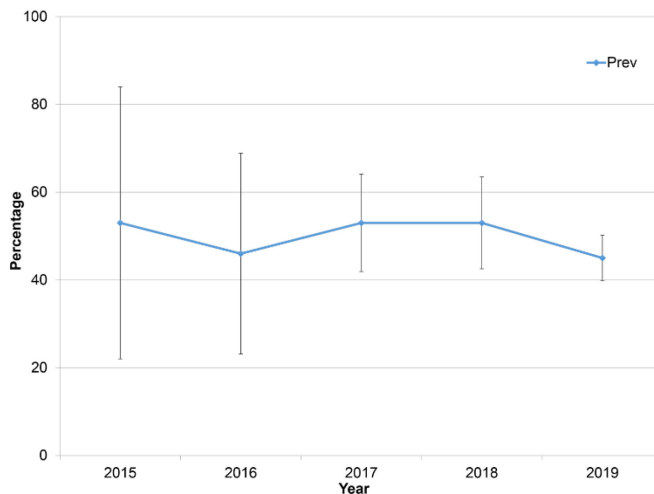
Between 2015 and 2019, 1,263 *A. baumannii* isolates were tested of which 632 (50.0%) isolates were CNS *A. baumannii*. The prevalence of CNS *A. baumannii* between 2015 and 2019 is presented in Figure 1. The percentage of CNS *A. baumannii* decreased from 53% in 2018 to 45% in 2019. However, statistical analysis indicated no statistically significant difference in the prevalence of CNS *A. baumannii* throughout the study period, $p = 0.160$ (Table 1).

The distribution of CNS *A. baumannii* and carbapenem-susceptible (CS) *A. baumannii* based on the types of ward and type of specimen are presented in Table 1. Our data suggested that CNS *A. baumannii* had different prevalence based on the type of ward ($p < 0.001$). CNS *A. baumannii* isolates obtained from the ICU had a higher prevalence than in non-ICU wards ($p < 0.001$). In the non-ICU and outpatient units, CS *A. baumannii* was more prevalent with $p < 0.001$ and $p = 0.033$, respectively.

Our data also suggested that there was a significant difference between CNS *A. baumannii* prevalence based on the type of specimen ($p = 0.001$) (Table 1). CNS *A. baumannii* was found more often in sputum than in non-sputum samples ($p = 0.009$). In contrast, CNS *A. baumannii* had less prevalent in urine samples compared to non-urine samples ($p = 0.002$).

The antimicrobial susceptibility patterns of *A. baumannii*, CS *A. baumannii*, and CNS *A. baumannii* are presented in Table 2. The antimicrobial susceptibility of all isolates (both CS and CNS *A. baumannii*) was high against amikacin, tigecycline, and trimethoprim/sulfamethoxazole, 78%, 73%, and 56%, respectively. The susceptibility of CNS *A. baumannii*

Figure 1. Prevalence of carbapenem non-susceptible *A. baumannii* at Arifin Achmad General Hospital between 2015-2019 (n = 1,263).



was lower compared to CS *A. baumannii* for all antibiotics. For example, only 64% of CNS *A. baumannii* were sensitive to amikacin while 93% of CS *A. baumannii* were sensitive to this antibiotic. Similarly, the susceptibility to tigecycline and trimethoprim/sulfamethoxazole were also lower among CNS *A. baumannii* than CS *A. baumannii*, 53% vs. 93% and 45% vs. 68%, respectively (Table 2).

Confirmation of *A. baumannii* and the presence of carbapenemase gene among CNS *A. baumannii* was conducted using duplex PCR targeting the *bla*_{OXA-51} and *bla*_{OXA-23}, respectively and the results are presented in Table 3. All isolates harbored the intrinsic *bla*_{OXA-51} gene, confirming the identification as *A. baumannii*. The *bla*_{OXA-23} was identified in 92% out of 24 tested CNS *A. baumannii* isolates.

Table 1. Distribution of carbapenem susceptible and non-susceptible *A. baumannii* based on unit source and specimen type (n = 1,263).

Variable	CNS <i>A. baumannii</i>	CS <i>A. baumannii</i>	Total	p-value
Year				0.160
2015	146 (53%)	128 (47%)	274 (100%)	
2016	124 (46%)	146 (54%)	270 (100%)	
2017	128 (53%)	115 (47%)	243 (100%)	
2018	131 (53%)	117 (47%)	248 (100%)	
2019	103 (45%)	125 (55%)	228 (100%)	
Type of ward				< 0.001
Intensive Care Unit	299 (68%)	142 (32%)	441 (100%)	< 0.001
Non-Intensive Care Unit	280 (40%)	418 (60%)	698 (100%)	< 0.001
Outpatient unit	38 (40%)	57 (60%)	95 (100%)	0.033
Others	15 (52%)	14 (48%)	29 (100%)	0.001
Specimen type				0.001
Pus	225 (48%)	244 (52%)	469 (100%)	0.259
Blood	67 (53%)	60 (47%)	127 (100%)	0.519
Sputum	316 (54%)	269 (46%)	585 (100%)	0.009
Urine	8 (24%)	26 (76%)	34 (100%)	0.002
Others	16 (33%)	32 (67%)	48 (100%)	0.001
Total	632 (50%)	631 (50%)	1263 (100%)	

CNS: carbapenem non-susceptible; CS: carbapenem susceptible.

Discussion

The prevalence of CNS *A. baumannii* varies among geographical locations and types of surveillance studies conducted. A study found that the prevalence of CNS *A. baumannii* was high globally ranging from 73% in South Africa to 97% in Mexico [15]. In Asia, the prevalence of CNS *A. baumannii* was approximately 40% in India [7], between 43–92% in Vietnam [7], between 17–98% in Nepal [7], and 50% in Singapore [16]. Some studies suggested an increase in CNS *A. baumannii* prevalence in some countries in Southeast Asia such as Malaysia [17,18], the Philippines [19], and Thailand [20]. The prevalence of CNS *A. baumannii* in our study significantly decreased from 53% in 2018 to 45% in 2019 after the Antimicrobial Stewardship Program (ASP) started in Arifin Achmad General Hospital in 2016. The ASP consisted of education as well as the establishment of antimicrobial guidelines and restrictions. The ASP might directly contribute to the decreased trend of CNS *A. baumannii* prevalence in the hospital.

Our data suggested that the prevalence of CNS *A. baumannii* was different based on the type of ward or unit. The samples from patients treated in the ICU had a higher CNS *A. baumannii* compared to those treated in non-ICU and outpatient units where the CS *A. baumannii* was more prevalent. It is not surprising since most of those patients treated in the ICU had a longer duration of stay in the hospital than other units and therefore had a higher chance to have a healthcare-associated infection. A systematic review and meta-analysis in Southeast Asian countries found that one of the most common microorganisms associated with healthcare-associated infections was *A. baumannii* [21]. A study found that some of the risk factors of CNS *A. baumannii* infection include having surgery, central catheter, tracheostomy, mechanical ventilation, enteral feeding, treatment with third-generation cephalosporin,

Table 3. Detection of *bla_{OXA-51}* and *bla_{OXA-23}* genes among CNS *A. baumannii* (n = 24).

PCR-result	<i>bla_{OXA-51}</i>	<i>bla_{OXA-23}</i>
Positive	24 (100%)	22 (92%)
Negative	0 (0%)	2 (8%)
Total	24 (100%)	24 (100%)

fluoroquinolone, or carbapenem antibiotics [22]. Eighteen years of data from a tertiary teaching hospital also found that the infection of CNS *A. baumannii* occurred more in the ICU patients [23].

Our data also suggested that the CNS *A. baumannii* was more prevalent in sputum and less in urine. In humans, *A. baumannii* could colonize the skin, wounds, respiratory, and gastrointestinal tracts [24]. Frequent clinical manifestations of *A. baumannii* infection are pneumonia, in particular ventilator-associated pneumonia (VAP) and sepsis [25]. It is possible that the CNS *A. baumannii* isolates obtained from VAP and/or sepsis patients in the ICU had the risk of being infected by resistant bacteria including CNS *A. baumannii*.

Antibiotic choices for infection caused by CS *A. baumannii* are ceftazidime, carbapenems, piperacillin/tazobactam, aminoglycosides, quinolones, or cefepime, either single or in combination [26]. Isolates in our current study showed poor susceptibility to these first-line antibiotics, less than 50%. The Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) in the USA in 2018, showed that 68.1% of *Acinetobacter* isolates were resistant to ceftazidime and 73.4% to ciprofloxacin [27]. The result was significantly higher than the data in 2017, which was 30% and 40%, respectively [27]. Treatment choice for *A. baumannii* resistant to those antibiotics becomes limited. Colistin becomes an option besides minocycline and tigecycline [26,28]. Our data did not include colistin and minocycline susceptibility due to

Table 2. Antibiotic sensitivity pattern of *A. baumannii*.

Antibiotic	<i>A. baumannii</i> (n = 1,263)	CNS <i>A. baumannii</i> (n = 632)	CS <i>A. baumannii</i> (n=631)
	Sensitive	Sensitive	Sensitive
Amikacin	78%	64%	93%
Ceftazidime	26%	1%	50%
Ciprofloxacin	34%	11%	56%
Ceftriaxone	4%	1%	7%
Cefazoline	0%	0%	1%
Cefepime	31%	5%	58%
Gentamycin	36%	12%	60%
Meropenem	50%	0%	100%
Ampicillin/sulbactam	44%	8%	81%
Trimethoprim/sulfamethoxazole	56%	45%	68%
Tigecycline	73%	53%	93%
Piperacillin/tazobactam	31%	7%	57%

CNS: carbapenem non-susceptible; CS: carbapenem susceptible.

the unavailability of both antibiotics in Indonesia. The high prevalence of CNS *A. baumannii* in our study highlights the need for a supply of colistin in the country. The susceptibility test is also needed due to the report of increasing colistin resistance observed in several studies [29].

A. baumannii has several antibiotic resistance mechanisms, mainly antibiotic hydrolysis by producing β -lactamase enzymes such as carbapenemase. The encoding gene of this enzyme can be native intrinsic such as *bla*_{OXA-51} or acquired such as *bla*_{OXA-23} [7]. Another resistance mechanism includes outer membrane protein (OMP) modification to reduce the permeability of antibiotics and to serve as a multi-drug efflux pump [7]. Our present study showed the prevalence of the *bla*_{OXA-23} gene was high (92%) among CNS *A. baumannii* isolates. The *bla*_{OXA-23} gene codes for class D carbapenemase, oxacillinase, which is the main carbapenemase found worldwide [10]. Several groups of carbapenemases have been identified in *A. baumannii* so far including metallo-beta-lactamases (VIM, IMP, and SIM) that were sporadically reported in several countries [10]. Recently *A. baumannii* carrying the NDM1 gene was also reported in India [10]. Further study is needed to detect those carbapenemase-related genes in Indonesia since only 92% of the tested CNS *A. baumannii* isolates harbored the *bla*_{OXA-23} gene.

There are some limitations of the study that need to be discussed. The number of the samples tested for molecular confirmation was relatively small; however, the prevalence of the *bla*_{OXA-23} gene among CNS *A. baumannii* isolates found in this study is similar to other studies in Indonesia including in Jakarta (91.8%) [30], Yogyakarta (82.4%) [31], and those from South and Southeast Asia countries [7]. The clinical diagnoses of the samples were not available from the database due to the IT system. Therefore, further comprehensive analysis for example to see the distribution of CNS *A. baumannii* based on clinical diagnoses is needed. In this study, the CNS *A. baumannii* was defined as *A. baumannii* that was not sensitive to the meropenem only and there were no sensitivity tests conducted imipenem, or doripenem. Therefore, CNS *A. baumannii* in this present study is a meropenem non-susceptible strain of *A. baumannii*.

Conclusions

CNS *A. baumannii* has become a problem in Arifin Achmad Hospital, Indonesia, particularly in patients treated in the ICU. Most CNS *A. baumannii* isolates are antimicrobial-resistant and have the highest

susceptibility to amikacin, tigecycline, and trimethoprim/sulfamethoxazole. The vast majority (92%) of the CNS *A. baumannii* isolates harbored the *bla*_{OXA-23} gene. Efficient implementation of infection prevention and control, as well as antibiotic stewardship programs, are required, in the hospitals, to tackle the growing multi-resistant *A. baumannii* in Indonesia.

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