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Increased Number of Metallo- or OXA Carbapenemase Producing *Acinetobacter baumannii* Isolated from Tangerang, Indonesia

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Abstract

Background: The escalation of Carbapenem-Resistant *Acinetobacter baumannii* (CRAB) has created a major issue in the antibiotic option for the patients admitted in intensive care unit globally, including in Indonesia. The aim of our study was to evaluate the presence of metallo- or OXA carbapenemase producing *A.baumannii* and their antibiotic susceptibility level in the clinical specimens.

Methods and Findings: During a 3 year-period, 117 isolates of *A.baumannii* were collected from patients admitted in the intensive care unit of Siloam General Hospital, Tangerang, Indonesia. All *A.baumannii* isolates were filtered for antibiotic susceptibility and their phenotypes regarding MICs of β -lactams class using VITEX-2 AES. Of the entire drugs tested, tigecycline had the highest level of sensitivity. Most of *A.baumannii* isolates in this study were highly resistant to meropenem and cephalosporins especially ceftriaxone. The prevalence of metallo- or OXA carbapenemase was 55.9% in 2013, 86.8% in 2014, and 62.2% in 2015.

Conclusion: The low number of drug susceptibility among *A.baumannii* observed in this study demonstrated multidrug-resistant resistant organism especially CRAB, The high level of metallo- or OXA carbapenemase phenotypes was associated with this findings.

pneumonia, meningitis, bacteremia, urinary tract infection, and wound infection with mortality rate 28-68% and prolonged length of hospital stay [3,4]. The use of medical equipment, such as mechanical ventilation, tracheostomy, and antibiotic treatment with third generation cephalosporins, fluoroquinolones, or carbapenems are a risk factors of getting acinetobacter infections, particularly MultiDrug-Resistant (MDR) *A.baumannii* [2,5].

In the recent years, the prevalence of MDR *A.baumannii* have been rapidly increasing and recognized as the most difficult antibiotic-resistant Gram-negative bacilli to treat and control [1,6].

The decreased susceptibility of various antibiotic among *A.baumannii* isolates have implicated in limited selection of therapeutic options for patients, mainly if the isolates were resistant to the carbapenems [1,6,7].

The production of class B Metallo- β -Lactamases (MBLs), such as Verona Integron (VIM) and Imipenemase (IMP) types, which can hydrolyze all β -lactams, except monobactams has been assured as the major mechanism for the spreading of resistance to *Acinetobacter* species [8,9]. Other carbapenemase enzymes, class D Oxacillinases (OXAs) with activity against carbapenems have been reported worldwide [10]. The MBLs or OXAs were a major threat for clinicians since they were easily transferred among bacteria and induced hospital acquired infection [9,11].

The finding of these enzymes is important in the field of infection control and public health perspective [3,12]. Unfortunately, the information of these bacterias and their mechanisms resistance among hospitalized patients in Indonesia is limited. Along with this mind, our study investigate the prevalence of metallo- or OXA carbapenemase producing *A. baumannii* and its antibiotic susceptibility pattern from clinical isolates in secondary care healthcare facility in Tangerang, Indonesia.

Keywords: Carbapenem-resistant *Acinetobacter baumannii*; Phenotypes; Tigecycline; Intensive care unit

Introduction

Acinetobacter baumannii (*A.baumannii*) is a Gram-negative non-fermenting bacilli, which is one of the most opportunistic pathogen in nosocomial infections, especially in critically-ill patients with poor immune response [1,2]. This bacteria frequently causes health care-associated infections, including

Materials and Methods

During the 3 year-period of January 2013 until December 2015, 117 consecutive non-duplicate clinical isolates of *A. baumannii* were collected from medical records of admitted patients in the ICU of Siloam General Hospital, Tangerang, Indonesia. Antibiotic susceptibility and mechanisms of resistance to beta lactams were retrieved from data system and converted into a format which was used for analysis. Data of antimicrobial susceptibility level were presented as number. Data were analyzed using SPSS version 21.

A.baumannii identification and susceptibility testing

The clinical isolate samples were obtained as a routine examination of admitted patients in the ICU. The collection and transport of specimens were conducted according to the standard protocol from our microbiology laboratory. All clinical samples were immediately inoculated onto standard MacConkey agar prepared in house from dehydrated MacConkey powder according to manufacturer's instructions and incubated at 37°C for 24 hours. Oxidase negative colonies morphologically similar to *Acinetobacter* were identified using an automated system GN-ID card from VITEX-2 Compact®. Susceptibility test was performed using the VITEX Antibiotic Susceptibility Testing (AST) N-100 card and results were

interpreted based on current Clinical and Laboratory Standard Institute (CLSI) guideline [13]. *Escherichia coli* ATCC® 25922 and *Pseudomonas aeruginosa* ATCC® 27853 were used as control strains, and *E. coli* ATCC® 35218 for β -lactam/ β -lactamase inhibitor combinations [13]. Detection of metallo- or OXA carbapenemase was provided by the Advance Expert System (AES) databases on phenotypes regarding Minimum Inhibitory Concentration (MICs) breakpoint of β -lactam classes [14,15].

Results

In the study period, a total of 954 positive bacterial cultures were collected from ICU, in which 117 (12.3%) were identified as *A. baumannii*. As it is shown in **Table 1**, most common source of infection was respiratory tract 104 (88.9%), while the least number proportion 2 (1.7%) was isolated from blood and urine samples.

Our study demonstrated an increased number of MBLs or OXAs phenotypes with Outer Membrane Protein (OMP) impermeability (**Table 1**). The highest number of these phenotypes was in 2014 with 33 (86.8%) events. In 2013 and 2015 the number of metallo- or OXA carbapenemase producing *A. baumannii* was 19 (55.9%) and 28 (62.2%) consecutively.

Table 1 Characteristics of *A. baumannii* isolates from clinical specimens

Characteristics	Year			Total
	2013	2014	2015	
	n = 34	n = 38	n = 45	n = 117
Type of specimens				
Sputum	31 (91.18%)	28 (73.68%)	41 (91.12%)	100 (85.47%)
Bronchial lavage	1 (2.94%)	2 (5.27%)	1 (2.22%)	4 (3.42%)
Blood	1 (2.94%)	3 (7.89%)	1 (2.22%)	5 (4.27%)
Cerebrospinal fluid	0 (0%)	2 (5.27%)	0 (0%)	2 (1.71%)
Abscess swab	0 (0%)	3 (7.89%)	1 (2.22%)	4 (3.42%)
Urine	1 (2.94%)	0 (0%)	1 (2.22%)	2 (1.71%)
Phenotype of beta lactams resistance				
Acquired penicillinase	14 (41.17%)	5 (13.15%)	11 (24.44%)	30 (25.64%)
Metallo- or OXA lactamase with resistant carbapenemase (impermeability)	19 (55.88%)	33 (86.84%)	28 (62.22%)	80 (68.37%)
High level resistance	2 (5.88%)	1 (2.63%)	4 (8.88%)	7 (5.98%)
Wild (cephalosporinase)	14 (41.17%)	5 (13.15%)	11 (24.44%)	30 (25.64%)

The isolates exhibited high sensitivity to amikacin (73.5%, 65.8%, and 66.7% in 2013, 2014, and 2015) and tigecycline (76.5%, 65.8%, and 71.1% in 2013, 2014, and 2014). Antibiotic that had moderate level of susceptibility was trimethoprim-

sulfamethoxazole (73.2%, 57.9%, and 66.7% in 2013, 2014, and 2015).

Meropenem, one of β -lactams class antibiotic demonstrated low sensitivity level to isolates for three consecutive years. Mostly *A.baumannii* isolates in this study were highly resistant

to cephalosporins, especially against ceftriaxone. This study noted a relation between MBLs or class OXAs with resistant carbapenemase impermeability phenotypes and MIC ≥ 16 against meropenem. The MICs level for various antibiotics was shown in **Table 2**.

Discussion

In this study, the prevalence of *A.baumannii* was found to be 12.3%. This result was higher than in other study, such it was made in Indonesia [16] (0.4%) and India [17] (7.5%). This study results was comparable with other study conducted by Tsakiridou et al. which found that hospitalization in ICU significantly increases the risk of *A.baumannii* ventilator-associated pneumonia [18].

Table 2 Distribution of minimum inhibitory concentrations (MICs) of various antibiotics for *A.baumannii* isolated from ICU

Antibiotics	MIC break point ($\mu\text{g/ml}$)		
	MIC range	Sensitive	Resistance
Ampicillin/sulbactam	≤ 2 - ≥ 32	≤ 2 - 4	≥ 32
Ceftazidime	4 - ≥ 64	04-Aug	≥ 64
Ceftriaxone	8 - ≥ 64	8	≥ 64
Cefepime	≤ 1 - ≥ 64	≤ 1 - 8	32 - ≥ 64
Piperacillin/tazobactam	≤ 4 - ≥ 128	≤ 4 - 16	≥ 128
Ciprofloxacin	≤ 0.25 - ≥ 4	≤ 0.25 - 5	≥ 4
Amikacin	≤ 0.25 - ≥ 16	≤ 0.25 - 4	≥ 16
Meropenem	≤ 2 - ≥ 64	≤ 2 - ≥ 16	≥ 64
Trimethoprim/sulfamethoxazole	≤ 20 - ≥ 320	≤ 2 - 40	160 - ≥ 320
Tigecycline	≤ 0.5 - ≥ 8	≤ 0.5 - 2	≥ 8

Factors that raised *A.baumannii* infections were parallel to other Gram-negative bacilli which were requiring mechanical ventilation, previous antibiotic consumption with third generation cephalosporins, fluoroquinolones, and carbapenems, or debilitated patients [5,19,20].

Almost all of isolates in our study were carbapenem-resistant *A.baumannii* (CRAB) which was associated to the ability of various enzymes that are produced, specifically MBLs and drug impermeability [6,21,22]. Studies reported that the β -lactams class antibiotic resistance mechanism is the result of carbapenemase and OMP impermeability [4,22,23]. Previous studies from different geographical regions found that MBLs production among *A.baumannii* strain were detected in the range of 49-90% [6-8,21-23]. Considerably high prevalence of isolates were MBLs positive Acinetobacter (68.4%) could be the major reason of high CRAB among *A.baumannii* isolates in this study. The proportion of MBLs positive *A.baumannii* isolates in this study was higher than prior study by Peymani et al. that found the proportion of 49%, emphasize its emergence [8]. The

increasing prevalence of MBLs producing in this study was accordance with other studies in Iran [8,20]. Other predominant mechanism of CRAB, such as OXA-23 or OXA-24 carbapenemase could also explain the high prevalence of MBLs production [23]. This circumstances was also related to the increased consumption or heavy use of carbapenems and ciprofloxacin in hospitals.

In the last decades, there were emergence *A.baumannii* infections in ICU worldwide including Indonesia. Antibiotic sensitivity pattern in the present study showed high resistance against third generation cephalosporins, ciprofloxacin, and meropenem. Other antibiotics tested such as tigecycline, amikacin, and trimethoprim-sulfamethoxazole showed better sensitivity level. In a relatively similar study, Dent et al. reported prevalence of *A.baumannii* susceptibility to amikacin was 58% [24]. A study carried out by Gonlugur et al. in Turkey found that the only antibiotic susceptible against *A.baumannii* is amikacin [7]. Studies in Saudi Arabia and Iran found that more than 90% of *A.baumannii* isolates was sensitive to tigecycline [25]. Analysis of susceptibility pattern in Asia and the Middle East described that *A. baumannii* had high resistance to various antibiotics class, such as cephalosporins, tetracycline, β -lactam/ β -lactam inhibitor combination, fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole [26-28]. This not only caused the high incidence of MDR phenotype but also Extensively Drug-Resistant (XDR) phenotype which kept increasing in the past decades [21]. The reason for disparity in susceptibility pattern and phenotype of *A.baumannii* is presumably due to variety in clinical samples, setting of studies, and empirical antibiotics treatment in each geographical regions [27]. Our findings on antibiotic susceptibility and phenotypic characterization showed no significant variance with other regions globally (**Figure 1**).

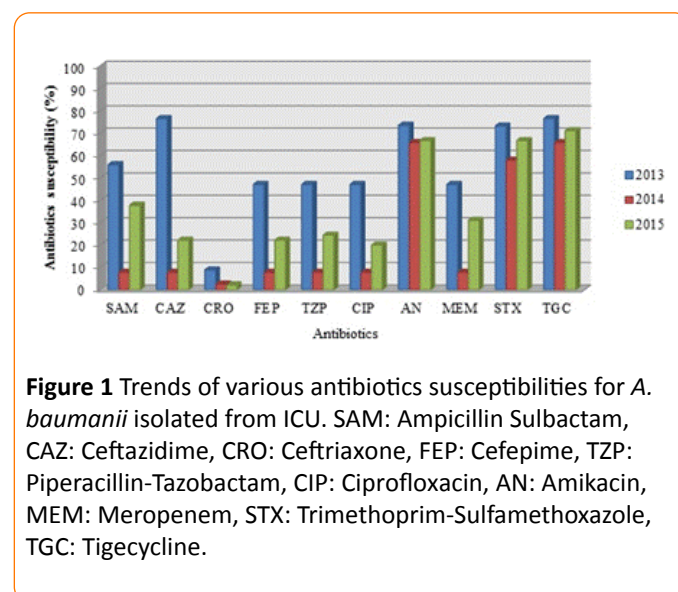


Figure 1 Trends of various antibiotics susceptibilities for *A. baumannii* isolated from ICU. SAM: Ampicillin Sulbactam, CAZ: Ceftazidime, CRO: Ceftriaxone, FEP: Cefepime, TZP: Piperacillin-Tazobactam, CIP: Ciprofloxacin, AN: Amikacin, MEM: Meropenem, STX: Trimethoprim-Sulfamethoxazole, TGC: Tigecycline.

Conclusion

The low number of drug susceptibility among *A.baumannii* strain observed in this study demonstrated MDR organism, especially CRAB. Thus, there were limited choices of antibiotic

available for empirical therapy in ICU setting, such as amikacin, tigecycline, or trimethoprim-sulfamethoxazole. The significantly high level of metallo- or OXA carbapenemase phenotypes was associated with the low susceptibility against meropenem and cephalosporins.

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