Detection of *bla*OXA genes and identification of biofilm-producing capacity of *Acinetobacter baumannii* in a tertiary teaching hospital, Klaten, Indonesia

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ABSTRACT

Introduction: *Acinetobacter baumannii (A. baumannii)* is commonly found as an agent of nosocomial infections and demonstrates a high antibiotic resistance due to its carbapenemase production. The objectives of this study were to explore the antibiotic resistance pattern, the presence of OXAs genes and the biofilm-producing capacity of *A. baumannii* isolated from clinical specimens.

Methods: Antibiotics susceptibility testing, detection of OXAs genes and the biofilm-producing capacity were performed using the Kirby Bauer method, polymerase chain reaction (PCR) and adherence quantitative assays, respectively.

Results: A total of 80 *A. baumannii* isolates were mainly obtained from sputum and most of them were resistant to antibiotics. All *A. baumannii* carried *bla*$_{OXA-51}$ gene, yet no *bla*$_{OXA-24}$ and *bla*$_{OXA-58}$ genes were detected. Fourteen (82.4%) of the 17 meropenem resistant isolates carried *bla*$_{OXA-23}$ gene, but it was not found in meropenem sensitive isolates. In addition, sixty (75.0%) of 80 isolates were biofilm producers with 2 (2.5%), 16 (20.0%), and 42 (52.5%) isolates were identified as strong, moderate and weak biofilm producers, respectively.

Conclusion: Most of *A. baumannii* isolates had a high level of antibiotic resistance and had a capacity to produce biofilm.

KEY WORDS:
A. baumannii, biofilm, antibiotic, resistance, OXA genes

INTRODUCTION

Acinetobacter baumannii (A. baumanii) is a Gram negative bacterium that commonly causes infections in hospitals. *Acinetobacter baumannii* is a commensal bacterium, which may become pathogenic and infects immunocompromised patients or patients with indwelling devices. *Acinetobacter baumannii* is known as the causative agent of ventilator-associated pneumonia, bacteremia, meningitis, urinary tract infections, skin and soft tissue infections, as well as central nervous system and bone infection.[1,2]

Some studies reported an increased resistance of *A. baumannii* to antibiotics. In the United States, it was reported that the resistance of *A. baumannii* to imipenem was 10% in 1999-2005 and increased to 48% in 2008. In the same period, the resistance to meropenem increased from 19% to 57.4%.[3] Nordmann et al.[4] reported that 47.1% and 45.2% of *A. baumannii* from various countries in Europe were resistant to imipenem and meropenem, respectively.

In Indonesia, the antibiotic resistance level of *A. baumannii* varies according to the regions. In Medan (North Sumatera), *A. baumannii* was identified in 17.4% of various clinical specimens; 23.0% of which were resistant to imipenem and meropenem.[5] In Jakarta (the capital city), 50.5% of *A. baumannii* were resistant to carbapenem.[6] While in Klaten (Central Java), among 59 *A. baumannii* isolates, 44.1% were resistant to meropenem.[7]

The high level of *A. baumannii* resistance to antibiotics is due to its ability to produce carbapenemase and to form biofilms.[8] Carbapenemase-producing bacteria should receive a special attention, since it is associated with the multi-drug resistance (MDR), which then leads to a more limited antibiotic choice. Thus, detection of carbapenemase-producing bacteria is an important aspect in clinical settings. However, the detection is challenging. While a mild increase in the value of the carbapenem's minimum inhibitory concentration could be detected, a molecular approach is required for its detection. Resistance to carbapenem is mediated by gene encoding oxacillinases (OXAs) and rarely via metallo-β-lactamases (MBLs).[9-11]

The increased incidence of *A. baumannii* infections, along with an increased level of antibiotic resistance inspired us to determine the sensitivity pattern of *A. baumannii* clinical isolates to various antibiotics, as well as the ability to encode carbapenemase and the capacity to produce biofilms in our hospital settings.

MATERIALS AND METHODS

Subject

This study was an observational study with a cross sectional design. The subjects of the study were *A. baumannii* isolates

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obtained from various clinical samples of patients hospitalized in Dr. Soeradji Tirtonegoro Hospital, Klaten (Central Java, Indonesia), a tertiary teaching hospital accommodating 405 beds, during March 2016 – February 2017.

Isolation, Identification and Antibiotic Susceptibility of *A. baumannii*

Acinetobacter baumannii was identified by culturing on McConkey agar, microscopic examinations with Gram staining and biochemical tests using Microbact (Oxoid, United Kingdom). Antibiotic susceptibility tests were performed by the Kirby Bauer method based on the Clinical and Laboratory Standards Institute.[12] *Acinetobacter baumannii* was classified as a multi-drug resistant *A. baumannii* (MDRAB) if the isolate was resistant to three or more different classes of antimicrobials.[13]

Detection of bla_{oxa} genes by polymerase chain reaction (PCR)

Genomic DNA was extracted from pure cultures using QIAamp DNA stool minikit (Qiagen, Canada) according to the manufacturer's instructions. The purified DNA was used for PCR to detect bla_{OXA} genes. For the detection of bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, and bla_{OXA-58} the following primers were used OXA-23F (5'-GAT CGG ATT GGA GAA CCA GA-3'), OXA-23R (5'-ATT TCT GAC CGC ATT TCC AT-3') with expected PCR product was 501 bp; OXA 24F (5'-GGT TAG TTG GCC CCC TTA AA-3'), OXA-24R (5'-AGT TGA GCG AAA AGG GGA TT-3') with expected PCR product was 246 bp; OXA-51F (5'-TAA TGC TTT GAT CGG CCT TG-3'), OXA-51R (5'-TGG ATT GCA CTT CAT CTT GG-3') with expected PCR product was 353 bp; OXA-58F (5'-AAG TAT TGG GGC TTG TGC TG-3') and OXA-58R (5'-CCC CTC TGC GCT CTA CAT AC-3') with expected PCR product was 599 bp, respectively.[14,15]

PCR reaction was conducted with the final volume of 25 µl containing DNA, ddH_2O, $(NH_4)_2SO_4$ - $MgCl_2$ 200 mM (Fermantas, St.Leon-Rot, Germany), $MgCl_2$ 25 mM (Fermantas, St.Leon-Rot, Germany), dNTP mix 100 nM (Fermantas, St.Leon-Rot, Germany), forward and reverse primer 50 pmol/ µl (Genetica Science, Singapore) and Taq polymerase 500 U (Fermantas, St.Leon-Rot, Germany). The PCR amplification was conducted with an initial denaturation temperature of 94°C for five minutes; 30 cycles of 94°C for 25 seconds, 57°C for 40 seconds, and 72°C for 50 seconds; and a final extension step at 72°C for five minutes. The amplified products were visualized on 1% agarose gel, containing ethidium bromide.

Biofilm formation assay

Biofilm formation assay was determined by using quantitative adherence assay.[16] Each isolate was cultured overnight in trypticase soy broth (TSB) at 37°C. Two µL of cell suspension was inoculated in sterile 96 well polystyrene microtitre plates with 198 µl of TSB. After 24 h of incubation at 37°C, the wells were gently washed three times with 200 µL of phosphate buffered saline (PBS), then dried in an inverted position and stained with 50 µL of 0.1% crystal violet. Subsequently, the wells were gently washed three times with 200 µL of distilled water and dried in an inverted position. The wells were rinsed again in 200 µL of 5% isopropanol acid to solubilize the residual crystal violet. The optical density (OD) at 570 nm was determined using microplate reader. Each isolate was tested by using several wells (repeated 8-12 wells), and the average optical density was obtained. Optical density cut-off (ODc) was defined as average OD of negative control + (3× standard deviation (SD) of negative control).

The following values were assigned for biofilm determination:[16]

- Non-biofilm producer: OD ≤ ODc
- Weak biofilm producer: 2×ODc < OD ≤4×ODc
- Medium biofilm producer: 2×ODc < OD ≤4×ODc
- Strong biofilm producer: OD > 4×ODc

Statistical analysis

The variables were described using frequencies and percentages. Chi-square tests were used to determine the association between the biofilm producing capacity of *A. baumannii* and the sensitivity pattern to antibiotics using STATA 13 software (STATA, College Station, Tex). The results were presented as prevelence ratios with a 95% confidence interval. P-values of less than 0.05 were considered statistically significant.

Ethical approval

The research protocol was approved by the Ethical Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (KE/FK/062/EC/2016).

RESULTS

Characteristics of clinical samples

During the period of March 2016 - February 2017, we examined 80 *A. baumannii* isolates from patients at Dr. Soeradji Tirtonegoro Central Referral Hospital, Klaten, Indonesia. The isolates were obtained from 52 male (65.0%) and 28 female (35.0%) patients. *Acinetobacter baumannii* isolates were mainly obtained from patients aged 17-65 years old (63.8%) (Table I). In this study, *A. baumannii* specimens were mainly isolated from sputum (46.3%) and swab of the wound (36.3%) (Table I).

Antibiotic susceptibility pattern and detection of bla_{oxa} genes

The majority of *A. baumannii* were resistant to a wide range of antibiotics. *Acinetobacter baumannii* had only a good sensitivity to amikacin (75.0%), ampicillin-sulbactam (75.0%), and meropenem (76.3%) (Table II).

Detections of bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, bla_{OXA-58} genes were conducted in 17 meropenem resistant and 4 meropenem sensitive *A. baumannii* isolates (Table III). Figure IA and IB showed the representative of PCR products of bla_{OXA-23} and bla_{OXA-51} genes that showed 501 bp and 353 bp of PCR product respectively. All *A. baumannii* isolates carried the bla_{OXA-51} gene. However, no bla_{OXA-24} and bla_{OXA-58} genes were detected in any of the *A. baumannii* isolates. Fourteen (82.4%) of the 17 meropenem resistant isolates carried bla_{OXA-23} gene. bla_{OXA-23} gene was not found in meropenem sensitive isolates (Table III).

Table I: Clinical characteristics of patients from which *A. baumannii* were isolated

Characteristics		Total	
		n	%
Sex	Male	52	65.0
	Female	28	35.0
	Total	80	100.0
Age (year)	0 - 17	4	5.0
	17 - 65	51	63.8
	>65	24	30.0
	unknown	1	1.3
	Total	80	100.0
Sampling location	Non-ICU	54	67.5
	ICU/ PICU/HCU/ICCU	26	32.5
	Total	80	100.0
Clinical Specimens	Sputum	37	46.3
	Swab of wound	29	36.3
	Blood	4	5.0
	Pus	4	5.0
	Bronchial washing	2	2.5
	Pleural fluid	2	2.5
	Tracheal aspiration	1	1.3
	Urine	1	1.3
	Total	80	100.0

Note: ICU = Intensive Care Unit; PICU = Pediatric Intensive Care Unit;
HCU = High Care Unit; ICCU = Intensive Cardiac Care Unit.

Table II: Antibiotic susceptibility pattern of *A. baumannii* clinical isolates

No	Antibiotics	Number tested	Sensitive		Resistant	
			n	%	n	%
1	Amikacin	80	60	75.0	20	25.0
2	Ampicillin-sulbactam	80	60	75.0	20	25.0
3	Gentamicin	80	45	56.3	35	43.8
4	Cefepime	34	14	41.2	20	58.8
5	Meropenem	80	61	76.3	19	23.8
6	Levofloxacin	80	36	45.0	44	55.0
7	Ceftriaxone	80	4	5.0	76	95.0
8	Tetracycline	74	32	43.2	42	56.8
9	Trimethoprim-sulfamethoxazole	76	51	67.1	25	32.9
10	Ciprofloxacin	40	8	20.0	32	80.0
11	Piperacillin-tazobactam	80	41	51.3	39	48.8
12	Tobramycin	80	41	51.3	39	48.8
13	Ceftazidime	80	30	37.5	50	62.5

Table III: Detection of *bla*oxa-23, *bla*oxa-24, *bla*oxa-51, *bla*oxa-58 genes of *A. baumannii* clinical isolates

No	Sample ID	Sensitivity to meropenem	Detection			
			*bla*OXA-23	*bla*OXA-24	*bla*OXA-51	*bla*OXA-58
1	16-0255	Resistant	Pos	Neg	Pos	Neg
2	16-0367	Resistant	Neg	Neg	Pos	Neg
3	16-0568	Resistant	Pos	Neg	Pos	Neg
4	16-0647	Resistant	Neg	Neg	Pos	Neg
5	16-0663	Resistant	Pos	Neg	Pos	Neg
6	16-0671	Resistant	Neg	Neg	Pos	Neg
7	16-0905	Resistant	Pos	Neg	Pos	Neg
8	16-0962	Resistant	Pos	Neg	Pos	Neg
9	16-1025	Resistant	Pos	Neg	Pos	Neg
10	16-1191	Resistant	Pos	Neg	Pos	Neg
11	16-1216	Resistant	Pos	Neg	Pos	Neg
12	16-1222	Resistant	Pos	Neg	Pos	Neg
13	16-1298	Resistant	Pos	Neg	Pos	Neg
14	16-1326	Resistant	Pos	Neg	Pos	Neg
15	17-0100	Resistant	Pos	Neg	Pos	Neg
16	17-0145	Resistant	Pos	Neg	Pos	Neg
17	17-0152	Resistant	Pos	Neg	Pos	Neg
18	17-0129	Sensitive	Neg	Neg	Pos	Neg
19	17-0137	Sensitive	Neg	Neg	Pos	Neg
20	17-0138	Sensitive	Neg	Neg	Pos	Neg
21	16-1251	Sensitive	Neg	Neg	Pos	Neg

Table IV: Association between *A. baumannii* multi drug resistance (MDR) and biofilm producing capacity

Classification		n	%	p-value	PR	CI 95 %
MDR	Biofilm producer	31	38.8	0.00	0.61	0.45-0.83
	Non biofilm producer	17	21.3			
Non-MDR	Biofilm producer	29	36.3			
	Non biofilm producer	3	3.8			
Total		80	100.0			

PR = prevalence ratio

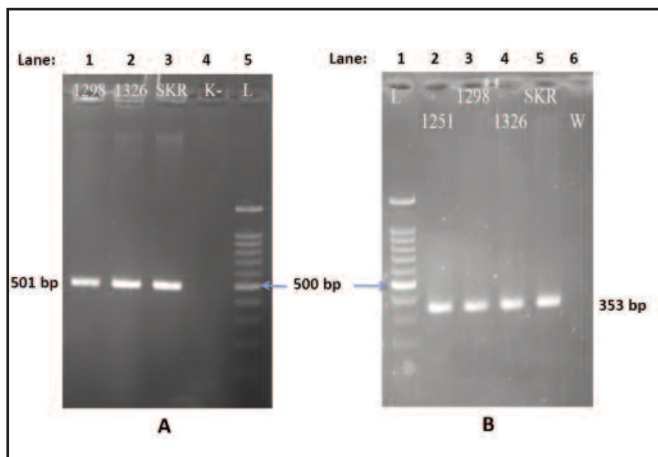

Fig. 1: Agarose gel electrophoresis (2%) used for separation of PCR products of bla_{OXA-23} gene (A) and bla_{OXA-51} gene (B). (A) Lane 1-3, samples from patients. Lane 4, negative control. Lane 5, DNA ladder. The expected product size of bla_{OXA-23} is 501 bp. (B) Lane 1: DNA ladder. Lane 2-5: samples from patients. Lane 6: negative control. The expected product size of bla_{OXA-51} is 353 bp.

Detection of biofilm formation

Based on quantitative adherence assay, among the 80 isolates tested, there were 2 (2.5%), 16 (20.0%), and 42 (52.5%) isolates identified as strong, moderate and weak biofilm producers, respectively. There were 20 (25.0%) isolates that were not biofilm producers. In this study, 48 *A. baumannii* isolates were found to be MDR and 32 isolates were non-MDR (Table IV). A statistical analysis using chi-square test showed a significant association between *A. baumannii* MDR and biofilm production capacity. Biofilm producing- *A. baumanni* isolates were less likely to develop MDR (PR = 0.61; 95% CI 0.45 - 0.83; $p < 0.05$) (Table IV).

DISCUSSION

Based on our study, amikacin, ampicillin-sulbactam, and meropenem were highly effective against *A. baumannii*. In contrast, *A. baumannii* was found to exhibit high resistance against gentamicin, cefepime, levofloxacin, ceftriaxone, tetracycline, ciprofloxacin, piperacillin-tazobactam, tobramycin, ceftazidime and trimethoprim-sulfamethoxazole. However, the results of this study were not consistent with the findings reported by Aulia et al.,[7] who conducted their study in the same hospital in 2012. They collected 59 *A. baumannii* isolates from various specimens, mostly were obtained from sputum (40.7%). The sensitivity of *A. baumannii* isolates to meropenem, amikacin, ampicillin-sulbactam, ciprofloxacin and ceftriaxone were 56.0%; 54.2%; 20.7%; 20.3% and 3.4% respectively. The inconsistency of results indicates that the resistance pattern of bacteria is changing over time and will need to be continuously monitored. Indeed, several studies of *A. baumannii* in Indonesia reported a different prevalence of carbapenem resistance. Studies conducted in Medan (North Sumatera), Jakarta (the capital city), Pekanbaru (Riau Province, Sumatera) and Surabaya (East Java) reported that 23.0%, 50.5%, 45.9% and 31.9% of *A. baumannii* isolates were resistant to carbapenem, respectively.[5,17-19]

Acinetobacter baumannii infections are difficult to treat, because of the high possibility to be resistant to several antibiotics. Carbapenem is the last option for treatment of multi-drug resistant *A. baumannii* (MDRAB). In this study, we found that 60.0% of *A. baumannii* were MDRAB. It is slightly lower than those reported by Aulia et al.[7] that showed 74.6% of *A. baumannii* isolates at Dr. Soeradji Tirtonegoro Klaten in 2012 were MDRAB.

Our studies are in line with several studies from other countries that demonstrated a high prevalence of MDR *A. baumannii*. The prevalence of MDR *A. baumannii* in USA, India and Brazil were 72% (177/247), 90.3% (65/72) and 100% (110/110), respectively. Management of MDR *A. baumannii* infections is a great challenge for physicians and clinical microbiologists. Its ability to survive in a hospital milieu and its ability to persist for extended periods of time in the environment makes it a frequent cause for healthcare-associated infections and it has led to multiple outbreaks.[20-22]

Carbapenem-resistance *A. baumannii* is mainly due to the ability to produce beta-lactamase encoded by oxacillinase genes.[23] Isolates of *A. baumannii* that were resistant to the carbapenem manifested by plasmid-encoded β-lactamases (OXA-23, OXA-24, and OXA-58). Every *A. baumannii* strain possessed a chromosomally encoded OXA β-lactamases (OXA-51), which could confer resistance to carbapenem when the genetic environment around the gene promoted its expression. In this study, bla_{OXA-51} gene was found in all *A. baumannii* isolates. However, no bla_{OXA-24} and bla_{OXA-58} genes were detected. Since bla_{OXA-51} gene is present in all strains of *A. baumannii*, this gene can be used as a positive control.[14] Therefore, all isolates in this study were confirmed as *A. baumannii* by genotypic (bla_{OXA-51}) detection, in line with the findings of worldwide studies.[24-29]

In this study, no bla_{OXA-24} and bla_{OXA-58} genes were found in all *A. baumannii* isolates, indicated that the carbapenemase resistance could possibly not due to these genes. This result is similar with the study conducted in Brazil[20] and the United

State of America.[29] In contrast, *bla*$_{OXA-24}$ gene was reported in some studies in Taiwan,[25] Iran,[26] Poland,[28] and France.[30] *bla*$_{OXA-58}$ gene was also found in some of *A. baumannii* isolates in Singapore,[24] France,[30] Germany,[31] Italy,[32] and Mexico.[33] Altogether, these results suggest a specific distribution of bla$_{OXA}$ gene variants in different regions.

Carvalho et al.[20] who conducted research in Brazil reported that 96 (87.3%) isolates of *A. baumannii* carried *bla*$_{OXA-23}$ gene. However, *bla*$_{OXA-14}$ and *bla*$_{OXA-58}$ were not detected. Zong et al.[34] reported that carbapenem resistant *A. baumannii* isolates in China carried *bla*$_{OXA-23}$ gene. We found that *bla*$_{OXA-23}$ gene was detected in most of meropenem resistant *A. baumannii*, but not detected in any meropenem sensitive isolates. These results support the data that, in addition to the antimicrobial-inactivating enzyme production, there are other mechanisms of antimicrobial resistance in *A. baumannii*. Such mechanisms include the reduced access to the bacterial targets (due to the decreased outer membrane permeability as a result of the loss or reduced expression of porins and overexpression of multidrug efflux pumps) and mutations that change the cellular targets (alterations in penicillin-binding proteins).[35]

The capacity to produce biofilm is an effective strategy to enhance survival during antibiotic treatment.[36] Biofilm producing-microorganisms can develop antimicrobial resistance by preventing the penetration of the antimicrobial agents through the biofilm matrix. Other mechanisms include altered growth rate and physiological conditions of biofilm organisms.[37] Surprisingly, our findings suggest that biofilm producer isolates were less likely to develop MDR. This result is in line with Qi et al. who reported that most of *A. baumanii* isolates with higher level of resistance tended to form weaker biofilms.[38] These results imply that biofilm acts as a mechanism for bacteria to get a better survival, especially in isolates with resistance level not high enough. This phenomenon was also observed in S. aureus where biofilm production was mostly found in MSSA isolates as compared to MRSA isolates.[39] In contrast, Gurung et al.[40] reported that antibiotic resistance was significantly higher among biofilm producer *A. baumanii* than that of non-producer. In our study, the biofilm producing capacity was tested in the planktonic cells of *A. baumannii*. Although the capacity of biofilm production in the form of planktonic cells may be different with that of biofilm cells, the result can be used as a caution to increase awareness of biofilm-producing *A. baumannii*.

Acinetobacter baumannii has a capacity to form biofilm on the catheter apparatus. Therefore, the use of urinary catheters and central venous catheters will increase the risk of catheter-associated urinary tract infection (CAUTI) as well as catheter-related bloodstream infections (CRBSIs). Furthermore, *A. baumannii* is capable of forming a biofilm on the surface of endotracheal tubes.[8]

LIMITATION OF STUDY

Antibiotics disk tested in this study did not encompass all antibiotics in carbapenem class due to some limited laboratory resources. Recent study conducted in Adam Malik Hospital Medan, Indonesia showed that the susceptibility of *A. baumannii* against several carbapenem antibiotics were comparable.[5] However, this study gave an important information about the resistance of *A. baumannii* to meropenem related to the resistance genes and their biofilm production.

CONCLUSIONS

Most of *A. baumannii* clinical isolates were resistant to antibiotics. Based on their sensitivity, amikacin, ampicillin-sulbactam, meropenem and trimethoprim-sulfamethoxazole can be considered for empiric therapy for *A. baumannii* infection. *bla*$_{OXA-51}$ gene was detected in all *A. baumannii* isolates, yet no *bla*$_{OXA-24}$ and *bla*$_{OXA-58}$ were detected. Most of meropenem resistant *A. baumannii* carried *bla*$_{OXA-23}$ gene. Since OXA genes can be efficiently transmitted by plasmid, it is important to prevent the spread of *bla*$_{OXA-23}$ gene to other bacteria to suppress the level of carbapenem resistance. Therefore, hospital infection control and antibiotic stewardship should be applied effectively.

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REFERENCES

1. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. Nat Rev Microbiol 2007; 5(12): 939-51.
2. Visca P, Seifert H, Towner KJ. Acinetobacter infection--an emerging threat to human health. IUBMB Life 2011; 63(12): 1048-54.
3. Rhomberg PR, Jones RN. Summary trends for the Meropenem Yearly Susceptibility Test Information Collection Program: a 10-year experience in the United States (1999-2008). Diagn Microbiol Infect Dis 2009; 65(4): 414-26.
4. Nordmann P, Picazo JJ, Mutters R, et al. Comparative activity of carbapenem testing: the COMPACT study. J Antimicrob Chemother 2011; 66(5): 1070-8.
5. Mayasari E, Siregar C. Prevalence of Acinetobacter baumannii isolated from clinical specimens in Adam Malik Hospital. Majalah Kedokteran Andalas 2014; 37: 1.
6. Gustawan IW, Satari HI, Amir I, Astrawinata DAW. Gambaran infeksi Acinetobacter baumannii dan pola sensitivitasnya terhadap antibiotika. Sari Pediatri 2014; 16: 35-40.
7. Aulia A, Nurrokhman, Mustafa M. Pola Kepekaan *A. baumannii* terhadap beberapa antibiotika di RS. Soeradji Tirtonegoro pada tahun 2012-2013. Bachelor thesis, Faculty of Medicine, Universitas Gadjah Mada 2014.
8. Longo F, Vuotto C, Donelli G. Biofilm formation in *Acinetobacter baumannii*. New Microbiol 2014; 37(2): 119-27.
9. Zarrilli R, Giannouli M, Tomasone F, Triassi M, Tsakris A. Carbapenem resistance in Acinetobacter baumannii: the molecular epidemic features of an emerging problem in health care facilities. J Infect Dev Ctries 2009; 3(5): 335-41.
10. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-beta-lactamases: a last frontier for beta-lactams? Lancet Infect Dis 2011; 11(5): 381-93.
11. Azimi L, Lari AR, Talebi M, Namvar AE, Jabbari M. Comparison between phenotypic and PCR for detection of OXA-23 type and metallo-beta-lactamases producer Acinetobacter spp. GMS Hyg Infect Control 2013; 8(2): Doc16.

12. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement. CLSI document M100-S25 2015.
13. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18(3): 268-81.
14. Brown S, Young HK, Amyes SG. Characterisation of OXA-51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacter baumannii* from Argentina. Clin Microbiol Infect 2005; 11(1): 15-23.
15. Woodford N, Ellington MJ, Coelho JM, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 2006; 27(4): 351-3.
16. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis 2011; 15(4): 305-11.
17. Karuniawati A, Saharman YR, Lestari DC. Detection of carbapenemase encoding genes in Enterobacteriaceae, Pseudomonas aeruginosa and *Acinetobacter baumannii* isolated from patients at Intensive Care Unit Cipto Mangunkusumo Hospital in 2011. Acta Med Indones-Indones J Intern Med 2013; 45: 101-6.
18. Anggraini D. Profile of *Acinetobacter baumannii* resistant meropenem in a private hospital in Pekanbaru, Riau Province, Indonesia from 2010 until 2015. Proceeding Annual Scientific Meeting, Perhimpunan Dokter Spesialis Mikrobiologi Klinik Indonesia (PAMKI) Solo, 10-11 September 2016.
19. Kurniawati ND, Widhyatmoko DB, Alimsardjono L. The antibiogram profile *Acinetobacter baumannii* in ward admission Dr. Soetomo General Hospital Surabaya. Proceeding Annual Scientific Meeting, Perhimpunan Dokter Spesialis Mikrobiologi Klinik Indonesia (PAMKI) Solo, 10-11 September 2016.
20. Carvalho KR, Carvalho-Assef AP, Peirano G, Santos LC, Pereira MJ, Asensi MD. Dissemination of multidrug-resistant Acinetobacter baumannii genotypes carrying bla(OXA-23) collected from hospitals in Rio de Janeiro, Brazil. Int J Antimicrob Agents 2009; 34(1): 25-8.
21. Badave GK, Kulkarni D. Biofilm Producing Multidrug Resistant *Acinetobacter baumannii*: An Emerging Challenge. J Clin Diagn Res 2015; 9(1): DC08-10.
22. Dent LL, Marshall DR, Pratap S, Hulette RB. Multidrug resistant *Acinetobacter baumannii*: a descriptive study in a city hospital. BMC Infect Dis 2010; 10: 196.
23. Fishbain J, Peleg AY. Treatment of Acinetobacter infections. Clin Infect Dis 2010; 51(1): 79-84.
24. Koh TH, Sng LH, Wang GC, Hsu LY, Zhao Y. IMP-4 and OXA beta-lactamases in *Acinetobacter baumannii* from Singapore. J Antimicrob Chemother 2007; 59(4): 627-32.
25. Kuo HY, Yang CM, Lin MF, Cheng WL, Tien N, Liou ML. Distribution of blaOXA-carrying imipenem-resistant Acinetobacter spp. in 3 hospitals in Taiwan. Diagn Microbiol Infect Dis 2010; 66(2): 195-9.
26. Azizi O, Shakibaie MR, Modarresi F, Shahcheraghi F. Molecular Detection of Class-D OXA Carbapenemase Genes in Biofilm and Non-Biofilm Forming Clinical Isolates of *Acinetobacter baumannii*. Jundishapur J Microbiol 2015; 8(1): e21042.
27. Villegas MV, Kattan JN, Correa A, et al. Dissemination of *Acinetobacter baumannii* clones with OXA-23 Carbapenemase in Colombian hospitals. Antimicrob Agents Chemother 2007; 51(6): 2001-4.
28. Nowak P, Paluchowska P, Budak A. Distribution of blaOXA genes among carbapenem-resistant *Acinetobacter baumannii* nosocomial strains in Poland. New Microbiol 2012; 35(3): 317-25.
29. Warner WA, Kuang SN, Hernandez R, et al. Molecular characterization and antimicrobial susceptibility of *Acinetobacter baumannii* isolates obtained from two hospital outbreaks in Los Angeles County, California, USA. BMC Infect Dis 2016; 16: 194.
30. Jeannot K, Diancourt L, Vaux S, et al. Molecular epidemiology of carbapenem non-susceptible *Acinetobacter baumannii* in France. PLoS One 2014; 9(12): e115452.
31. Schleicher X, Higgins PG, Wisplinghoff H, Korber-Irrgang B, Kresken M, Seifert H. Molecular epidemiology of *Acinetobacter baumannii* and Acinetobacter nosocomialis in Germany over a 5-year period (2005-2009). Clin Microbiol Infect 2013; 19(8): 737-42.
32. Principe L, Piazza A, Giani T, et al. Epidemic diffusion of OXA-23-producing *Acinetobacter baumannii* isolates in Italy: results of the first cross-sectional countrywide survey. J Clin Microbiol 2014; 52(8): 3004-10.
33. Bocanegra-Ibarias P, Pena-Lopez C, Camacho-Ortiz A, et al. Genetic characterisation of drug resistance and clonal dynamics of *Acinetobacter baumannii* in a hospital setting in Mexico. Int J Antimicrob Agents 2015; 45(3): 309-13.
34. Zong Z, Lu X, Valenzuela JK, Partridge SR, Iredell J. An outbreak of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemase in western China. Int J Antimicrob Agents 2008; 31(1): 50-4.
35. Manchanda V, Sanchaita S, Singh N. Multidrug resistant acinetobacter. J Glob Infect Dis 2010; 2(3): 291-304.
36. Rao RS, Karthika RU, Singh SP, et al. Correlation between biofilm production and multiple drug resistance in imipenem resistant clinical isolates of *Acinetobacter baumannii*. Indian J Med Microbiol 2008; 26(4): 333-7.
37. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002; 15(2): 167-93.
38. Qi L, Li H, Zhang C, et al. Relationship between antibiotic resistance, biofilm formation and biofilm-specific resistance in *Acinetobacter baumannii*. Front Microbiol 2016; 7: 483.
39. Nuryastuti T, Praseno P, Mustafa M. Preliminary study of biofilm formation properties and antibiotic susceptibility pattern of MRSA and MSSA isolates obtained in Yogyakarta, Indonesia. Mal J Microbiol 2015; 11(4): 383-390.
40. Gurung J, Khyriem AB, Banik A, et al. Association of biofilm production with multidrug resistance among clinical isolates of *Acinetobacter baumannii* and Pseudomonas aeruginosa from intensive care unit. Indian J Crit Care Med 2013; 17(4): 214-218.