Artikel Penelitian

PREVALENCE OF *ACINETOBACTER BAUMANNII* ISOLATED FROM CLINICAL SPECIMENS IN ADAM MALIK HOSPITAL

Evita Mayasari, Cherry Siregar

Abstrak

Acinetobacter baumannii merupakan spesies *Acinetobacter spp*. tersering diisolasi dari manusia, dan lebih sering dijumpai pada infeksi nosokomial dibandingkan dengan infeksi di komunitas. Eksistensi bakteri ini di lingkungan terkait dengan keragaman reservoir, kemampuan memperoleh gen pembawa sifat resisten antimikroba, dan sifat resisten terhadap pengeringan. Infeksi disebabkan strain *A.baumannii* yang resisten terhadap banyak antibiotik tidak mudah dikendalikan dan menjadi permasalahan di berbagai negara. Penelitian ini bertujuan untuk mengetahui prevalensi *A.baumannii* dari spesimen klinis di instalasi mikrobiologi klinik RSUP Haji Adam Malik serta pola kepekaannya terhadap berbagai antibiotik. Identifikasi dan uji kepekaan menggunakan mesin otomatis Vitek 2 dengan Advanced Expert System (AES). Penelitian ini menemukan 644/3693 (17,44%) isolat *A.baumannii* dari berbagai spesimen klinis. *A.baumannii* paling banyak diisolasi dari spesimen dahak. Penelitian ini menemukan 147/644 (23%) bahwa isolat carbapenem-resistent *A.baumannii* (imipenem dan meropenem). Sebagian besar isolat sensitif terhadap colistin, amikacin dan tigecycline. Prevalensi *A.baumanni* yang ditemukan pada penelitian ini adalah rendah namun resistensinya tinggi terhadap antibiotik terutama golongan penicillin, cephalosporin dan fluoroquinolon.

Kata kunci: *A. baumannii,* spesimen klinis, pola kepekaan, carbapenem-resistent *A.baumannii*

Abstract

Acinetobacter baumannii is the most frequent species of Acinetobacter spp. isolated from humans and more common in nosocomial infection than it is in community acquired infection. A.baumannii existence in environment is associated with the diversity of its reservoirs, its capacity to accumulate genes of antimicrobial resistence, and its resistence to desiccation. Infection of Multidrug resistent (MDR) strain of A.baumannii is not easy to manage and it has become a problem in many countries. The aim of this retrospective study was to investigate the prevalence of A.baumannii from routine clinical specimens sent to clinical microbiology laboratory RSUP HAM Medan and its susceptibility pattern to various antibiotics. Identification and susceptibility testing of A. baumannii was performed by Vitek 2 with Advanced Expert System (AES). A total of 644/3693 (17.44%) A.baumannii isolates were identified from various clinical specimens. From those isolates, there were 147 (23%) isolates of carbapenem-resistent A.baumannii (imipenem and meropenem). A.baumannii mainly isolated from sputum specimens, and most isolates were highly sensitive to colistin, amikacin and tigecycline. Low prevalence of A.baumannii was found in this study. However, the isolates showed high resistence level to antibiotics, particularly penicillin, cephalosporin and fluoroquinolones.

***Keywords**: A.baumannii, clinical specimens, susceptibility pattern, carbapenem-resistent A.baumannii*

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INTRODUCTION

Acinetobacter are opportunistic Gram-negative bacilli that occasionally appear round on smears (so termed cocobacilli).[1] These bacterias are widely distributed in soil and water. They can occasionally be cultured from skin, mucous membranes, secretions, and hospital environment. A.baumannii is the species most commonly isolated. A baumannii has been isolated from blood, sputum, skin, pleural fluid, and urine, usually in device associated infections. Acinetobacter encountered in nosocomial pneumonia often originates in the water of room humidifiers or vaporizers. In patients with A.baumannii bacteremia, intravenous catheters are the main source of infection. In patients with burns or with immune deficiencies, acinetobacter acts as an opportunistic pathogen and can produce sepsis.[2]

Acinetobacter strains are often resistent to antimicrobial agents, and therapy of infection can be difficult. They respond most commonly to gentamicin, amikacin, or tobramycin and to newer penicillins or cephalosporins.[2] To treat serious *A. baumannii* infections, carbapenems has been considered for many years as the drug of choice. Until recently, the majority of clinical *A. baumannii* isolates had been sensitive to the carbapenems. Unfortunately, the rapidly escalating prevalence of carbapenem -resistent *A.baumannii* (CRAB) in many parts of the world in the past few years has undermined the reliability of carbapenems.[3] *A.baumannii* resistence to carbapenems mediated by the lack of drug penetration (i.e., porin mutations and efflux pumps) and/ or carbapenem-hydrolyzing beta-lactamase enzymes such as OXA carbapenamases and metallo-beta-lactamases (MBLs).[4,5]

Recently, a large number of class D OXA-type enzymes which against carbapenems were characterized in locations that include Scotland, Spain, France, Japan, Singapore, China, Brazil, Cuba, and Kuwait.[6,7] Some *Acinetobacter* strains express class B MBLs, which hydrolyze a broad array of antimicrobial agents, including carbapenems. MBLs pose a significant threat because they are often located on mobile genetic elements easily transferred among bacteria.[7-9] Carbapenem-resistent isolates of *A.baumannii* are usually resistent to all classes of antimicrobials, and show intermediate resistence to rifampin, while usually retaining susceptibility to tigecycline and colistin.[4,10] Susceptibility testing should be done to help select the best antibiotics for therapy.[2]

Carbapenem resistence in *A. baumannii* is now an emerging issue worldwide. Surveillance studies indicate that the percentage of CRAB isolates gradually increased over the last ten years in Europe, North and Latin America. Numerous outbreaks of CRAB were reported from hospitals in Northern Europe, Southern Europe and the Middle East, North and Latin America, Tunisia, South Africa, China, Taiwan, Singapore, Hong Kong, Japan, South Korea and Australia.[4,10] Outbreaks caused by CRAB have also been observed in developing countries such as Morocco, Thailand, India and Indonesia.[10,11] At present, the article of CRAB prevalence in Indonesia is limited. A study in Sanglah General Hospital Bali reported that of all *A. baumannii* isolates, almost 40% was multidrug-resistent and three of them produced carbapenemase.[12] A study in internal medicine ward of dr. Soetomo Hospital Surabaya reported that 9,8% *A.baumannii* isolated from 467 urine specimens and antibiotics were potent towards these isolates.[13] In an effort to provide information regarding prevalence and antibiotic susceptibility pattern of *A.baumannii* isolated from clinical specimens of a hospital in Medan, we conducted this study.

METHODS

We conducted a retrospective study on various clinical specimens sent to clinical microbiology laboratory RSUP HAM, January until December 2012. Identification and susceptibility testing of *A. baumannii* were performed by Vitek2 system with Advanced Expert System (AES). Vitek2 AES is able to predict the presence of carbapenemases (metallo- or OXA) in *A. baumannii*. The entire beta lactam minimum inhibitory concentration (MIC) profile was taken into account and analysed by the AES software, providing the capability to flag isolates as potential carbapenemase producers, even those producing a low-level of enzyme.[14]

Except for sterile fluids, the clinical specimens were cultured in blood agar and MacConkey agar, incubated overnight at 37°C. The sterile fluids were put in Fan

Aerob Culture Bottles (BacT/ALERT), and incubated at 37°C for five days. The culture bottles with bacterial growth were proceeded for Gram stain and primary isolation in blood agar and MacConkey agar, incubated overnight at 37°C. The pure colonies from cultured isolates were picked and mixed with three milliliters of sterile saline (0.45% NaCl), prepared for inoculum suspension. Two tubes were prepared for each isolate. The inoculum turbidity was adapted with the turbidity of 0.5 McFarland standard using Den-sichek. First tube was placed in cassette for identification and second tube was placed for sensitivity test. A suitable Vitek2 card (GN/AST-N089/100) was placed according to instruction from *bioMérieux.* The antibiotics used for sensitivity test in this study included ampicillin, amoxicillin/ clavulanic-acid, ampicillin-sulbactam, meropenem, ciprofloxacin, ceftazidime, cefepime colistin, ceftriaxone, cefotaxime, gentamicin, imipenem, trimethoprim/sulfametoxazol, tobramycin, levofloxacin, tigecycline, amikacin, dan piperacillin-tazobactam.

RESULTS AND DISCUSSION

There are three most clinically relevant species of *Acinetobacter* that have been implicated in the vast majority of both community-acquired and nosocomial infections, i.e., *A.baumannii*, *Acinetobacter* genomic species 3 and *Acinetobacter* genomic species 13TU. According to the taxonomy, these organisms are classified as the *A.calcoaceticus-A.baumannii c*omplex, together with *A.calcoaceticus* that have frequently been recovered from soil and water but has never been implicated in serious clinical disease. The three clinically relevant members of the *A. calcoaceticus-A. baumannii* complex cannot be separated by currently available commercial identification systems. In fact, *A. baumannii*, *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU are uniformly identified as *A.baumannii* by the most widely used identification systems (including Vitek2). The term *A.baumannii* group for these species instead of *A. calcoaceticus-A.baumannii* complex reflects the fact that *A. baumannii*, *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU share important clinical and epidemiological characteristics and also eliminates the confusion resulting from inclusion of an environmental species, *A.calcoaceticus*.[4] Consistent with Vitek2 results, we simply use the species name *A.baumannii*.

A study revealed that Vitek2 cards were promising for rapid identification and reliable antibiotic susceptibility test (AST) for most species of nonglucosefermenting Gram-negative bacilli (including *Acinetobacter* spp.).[15] A study stated that Vitek2 method was the only automated susceptibility method that satisfied Food and Drug Administration (FDA) criteria for approval when used for imipenem and meropenem.[16] The latter result is consistent with the former that observed a very major errors (VME) rate of 0.7% for the Vitek2 instrument.[15,16] Therefore we supposed that our data are reliable enough to describe *A.baumannii* prevalence. We successfully identified a total of 644/3693 (17.44%) isolates of *A.baumannii* from various clinical specimens over the period January-December 2012 (Figure 1).

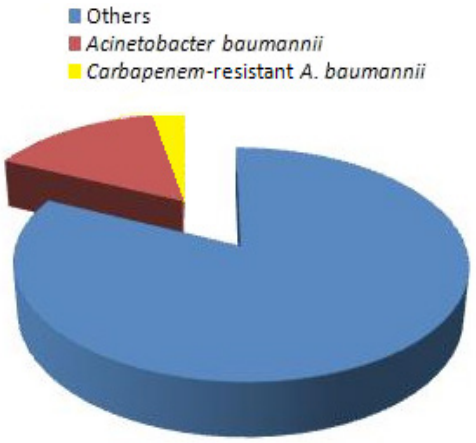

Figure 1. *A.baumannii* Prevalence in Total Bacterial Isolates

Vitek2 is able to determine the MIC and the production of carbapenemase during one test cycle. It detects metabolic changes by fluorescence based methods and identifies bacteria by monitoring the kinetics of bacterial growth. The MIC phenotype detected for the test isolate is interpolated with all the patterns of the database and the best is identified.[17] We found 147/644 (23%) CRAB (imipenem and meropenem). Because the problems of

false resistence and false susceptibility with imipenem and meropenem may occur, the detection of CRAB strains by Vitek2 should be confirmed by an additional test[14,18] (e.g. modified Hodge test, PCR), which we did not perform in this study. Therefore, our result regarding CRAB prevalence in *A.baumannii* isolat remain as a prediction.

In most institutions, the majority of *A.baumannii* isolates are from the respiretory tracts of hospitalized patients.[4] A study from South Africa revealed that from 232 CRAB complex isolates, 149 (64.2%) of the specimens were from endotracheal aspirates.[19] Recent study from Turkey revealed that from 74 strains isolated and identified as *A. baumannii*, most of the strains (46%) were isolated from tracheal aspirate specimens, and the study results is correspond to three other similar studies from Turkey.[20] We found *A.baumannii* isolates in sputum 59,94%, pus 12,35%, urine 6,02%, blood 4,07%, cerebrospinal fluid (CSF) 2,11%, pleural fluid 0,9%, bronchoalveolar lavage (BAL) 0,3% and other specimens 14,31%, as shown in Figure 2. Most of *A.baumannii* were isolated from respiratory tract (sputum), therefore our result is consistent with previous studies.

Antibiotic susceptibility pattern of *A.baumannii* isolates to some tested antibiotics is describe in table 1. Those isolates were sensitive to colistin (95.86%), amikacin (76.23%), tigecycline (75%), imipenem (63.39%), meropenem (62.43%), ampicillin-sulbactam (57.35%), tobramycin (53.54%) and trimethoprim/sulfamethoxazole (51.55%).

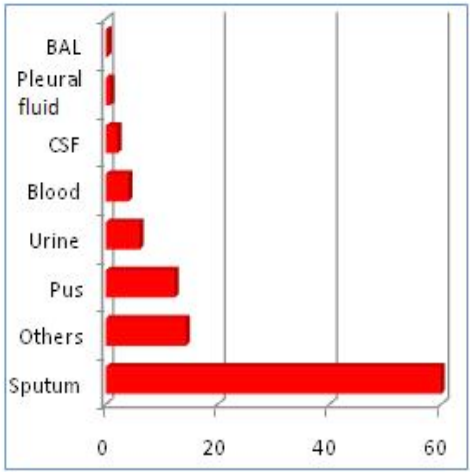

Figure 2. Distribution of *A.Baumannii* Isolates According to Clinical Specimens.

Table 1. Antibiotic Susceptibility Pattern of *A.Baumannii* isolates

Antibiotic Tested	No. tested	No. sensitive	% sensitive
Amikacin (AN)	551	420	76.23
Amoxicillin-clavulanic acid (AMC)	508	1	0.2
Ampicillin (AM)	644	0	0
Ampicillin-sulbactam (SAM)	136	78	57.35
Cefepime (FEP)	644	242	37.58
Cefotaxime (CTX)	508	85	16.73
Ceftazidime (CAZ)	644	227	35.25
Ceftriaxone (CRO)	136	13	9.56
Ciprofloxacin (CIP)	644	240	37.26
Colistin (CS)	508	487	95.86
Gentamicin (GM)	644	263	40.84
Imipenem (IPM)	508	322	63.39
Levofloxacin (LEV)	644	250	38.82
Meropenem (MEM)	551	344	62.43
Piperacillin/tazobactam (TZP)	642	248	38.63
Tigecycline (TGC)	644	483	75
Tobramycin (TM)	508	272	53.54
Trimethoprim/sulfamethoxazole (SXT)	644	332	51.55

In this study, the sensitivity test results of *A.baumannii* towards ampicillin-sulbactam, imipenem, tobramycin meropenem, trimethoprim/ sulfamethoxazole were tolerable. Ampicillin-sulbactam or a carbapenem (imipenem or meropenem) is adequate for the treatment of infections caused by antibiotic-susceptible strains of *Acinetobacter* spp.[21] A study of *A. baumannii* bacteremia in patients who presented with critical illness found that patiens with empirical combination therapy (e.g., carbapenem plus sulbactam or an aminogycoside) had a lower mortality rate than patients with monotherapy.[22] We found high (76.23%) amikacin-sensitive *A. baumannii* isolates, so this recommendation may be important for critical patients at risk of *A. baumannii* infections or in the clinical settings of the presence of MDR *A. baumannii*.[22]

Among the antibiotics that are considered as agents against MDR *A. baumannii*, tigecycline has received significant attention. Where the dissemination of MBLs accounts for the increasing prevalence of CRAB, the combination of colistin and rifampin with or without tigecycline should be considered. But there have been reports of *A. baumannii* resistence to tigecycline.[21] In this study, we found 25% tigecycline-resistent *A. baumannii* isolates. We found those isolates were most sensitive to colistin (95.87%), and our result is correspond to the previous studies from India[23] and Morocco.[24] When CRAB is suspected, intravenous colistin combined with rifampin and imipenem is a recommended therapy.[21] However, the optimal empirical combination regimens directed against *A. baumannii* infections should be based on local antimicrobial susceptibility patterns.[22]

A. baumannii rate of carriage by hospitalized patients is high. It causes a variety of nosocomial infections, comprising of bacteremia, urinary tract infection, surgical site infection and ventilator-associated pneumonia, mainly in intensive care unit (ICU) patients. It has emerged as a major cause of hospital acquired infections and unfortunately, some of them are MDR strains which probably caused by extensive use of antimicrobial agents. *A. baumannii* has a broad range of resistence mechanisms to all existing antibiotic classes and a capacity to acquire new determinants of resistence.[4,10,19,25] Genome sequence analysis of six *A. baumannii* clinical strains has shown the presence of a resistence island with a variable composition of resistence genes interspersed with transposons, integrons, and other mobile genetic elements in three of them. Plasmids carrying resistence genes involved in horizontal gene transfer have been described in *A. baumannii* strains.[10,26,27] A study showed that infections with MDR *Acinetobacter* are associated with length of hospitalization and its mortality rate higher than patients with infection by susceptible *Acinetobacter*.[28]

Within the last five years, the increasing number of CRAB is of major importance in the context of resistence to beta lactams.[24] Between 1998 and 2004, this rate of resistence in Europe, North America, South America, and Asia ranged between 0% and 40%.[21] But in most studies after 2005, the rate of resistence was greater than 50%. High rates of resistence were found in India (90%), Morocco (74%), Iran (63%), Italy (62,5%), China (55,6%), Turkey (53,7%), Korea (51%),[23,24,29-33] A study in ICU Cipto Mangunkusumo Hospital Jakarta found high rate of resistence (50.5%).[34] Sanglah Hospital Bali showed a moderate rate of resistence (36,9%).[12] To compare CRAB prevalence with previous studies, we still need confirmation of Vitek2 CRAB results by additional test (e.g.,modified Hodge test).

CONCLUSION

We found low prevalence of *A.baumannii,* and most of them are from sputum. Those isolates showed high resistence level to antibiotics, particularly penicillin, cephalosporin and fluoroquinolones. We detected CRAB prevalence from the isolates, but we need to perform additional tests in further studies to confirm the result. Antibiotic susceptibility pattern presented in this study may help clinicians to select appropriate empirical antimicrobial therapy for *A. baumannii* infections.

REFERENCES

1. Ryan KJ, Ray CG. Sherris Medical Microbiology: an Introduction to Infectious Diseases, 4th ed. New York; McGraw Hill, 2004.
2. Brooks GF, Butel JS, Carroll KC, Morse SA. Jawetz, Melnick & Adel-berg's Medical Microbiology. 24th ed. New York; McGrawHill, 2007.
3. Wu TC. Carbapenem-resistent or MDR *A.baumannii* - a clinician's perspective. Hong Kong Med Diary 2011;16(4):6-8.
4. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 2008;21(3):538-82.
5. Amudhan SM, Sekar U, Arunagiri K, Sekar B. OXA beta lactamase mediated CRAB. Indian J Med Microbiol 2011;29:269-74.
6. Fzal-Shah M, Woodford N, Livermore DM. Characterization of OXA-25, OXA-26 & OXA-27, molecular class D beta-lactamases associated with carbapenem resistence in clinical isolates of *A.baumannii.* Antimicrob Agents Chemother 2001;45:583–8.
7. Maragakis LL, Perl TM. *A.baumannii:* epidemiology, antimicrobial resistance and treatment options. Clin Infect Dis 2008;46:1254-63.
8. Bonomo RA, Szabo D. Mechanisms of MDR in *Acinetobacter* species and *Pseudomonas aeruginosa.* Clin Infect Dis.2006; 43(Suppl 2):49–56.
9. Thomson JM, Bonomo RA. The threat of antibiotic resistence in Gram-negative pathogenic bacteria: Beta-lactams in peril! Curr Opin Microbiol 2005;8:518–24.
10. Zarrilli R, Giannouli M, Tomasone F, Triassi M, Tsakris A. Carbapenem resistance in *A. baumannii*: the molecular epidemic features of an emerging problem in health care facilities. J Infect Dev Ctries 2009;3(5):335-41.
11. Mendes RE, Bell JM, Turnidge JD, Castanheira M, Jones RN. Emergence and widespread dissemination of OXA-23,-24/40 and -58 carbapenemases among *Acinetobacter* spp. in Asia-Pacific nations. J Antimicrob Chemother 2009;63:55-9.
12. Darwinata AE, Tarini NMA, Handayani L. Detection of carbapenemase by Modified Hodge test, distribution, resistence pattern of MDR *A.baumannii* in Sanglah General Hospital. 8th Nat Congress Indonesian Society Clin Microbiol; Denpasar Indonesia 1-2 Nov 2012.
13. Rifiana H,Retnoningsih D, Wasito EB. Antibiotic Sensitivity pattern of *A.baumannii* from urine specimens in internal medicine ward dr. Soetomo Hospital Surabaya. 8th Nat Congress Indonesian Society Clin Microbiol; Denpasar Indonesia 1-2 Nov 2012.
14. Anonym. BioMérieux Connection: Antibiotic resistence due to carbapenemases. Oct 2006;3(4). URL:http://bio merieuxusa.com/ upload/v3n4-1.pdf
15. Hsieh WS, Sung LL, Tsai KC, Ho HT. Evaluation of the Vitek2 cards for identification and antimicrobial susceptibility testing of non-glucosefermenting Gram-negative bacilli. APMIS 2009;117(4):241–7.
16. Markelz E, Mende K, Murray CK, et. al. Carbapenem Susceptibility Testing Errors using three automated systems, DD, Etest & Broth Microdilution and Carbapenem Resistence Genes in Isolates of *A. baumannii-calcoaceticus* Complex. Antimicrob Agents Chemother 2011;55(10): 4707-11.
17. Kottahachchi J, Faoagali J, Kleinschmidt S. Comparison of Meropenem MIC by E Test and VITEK 2 in resistent Pseudomonas & Acinetobacter isolates. Sri Lanka Journal of Infectious Diseases 2012;1(2):28-35.
18. Winstanley T, Courvalin P. Expert systems in clinical microbiology. Clin Microbiol Rev 2011;24(3):515-56.
19. Ahmed NH, Baba K, Clay C, Lekalakala R, Hoosen AA. In vitro activity of tigecycline against clinical isolates of CRAB complex in Pretoria, South Africa. BMC Research 2012;5:215.
20. Ağca H. Antibiotic susceptibilities of *A. baumannii* strains isolated from clinical samples. J Clin Anal Med 2013;4(1):48-50.
21. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of MDR *A.baumanni*. Antimicrob Agents Chemother 2007;51(10):3471–84.
22. Lee NY,Lee JC,Li MC,Li CW, Ko WC. Empirical antimicrobial therapy for critically ill patients with *A.baumannii* bacteremia: combination is better. J Microbiol, Immunol and Infect 2013;46(5):397-8.
23. Jaggi N, Sissodia P, Sharma L. *A.baumannii* isolates in a tertiary care hospital: antimicrobial resistence & clinical significance. JMID 2012;2(2):57-63.

24. Kabbaj H, Seffar M, Belefquih B. Prevalence of metallo beta lactamases-producing *A.baumannii* in a Moroccan hospital. ISRN Infect Dis 2013. URL: http://dx. doi. org/10.5402/2013/154921
25. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: MDR *A. baumannii*. Nat Rev Microbiol 2007;5:939-51.
26. Adams MA, Goglin K, Molyneaux N, et al. Comparative genome sequence analysis of MDR *A.baumannii*. J Bacteriol 2008;190(24):8053-64.
27. Poirel L, Nordmann P. CRAB: mechanisms and epidemiology. Clin Microbiol Infect 2006;12:826-36.
28. Sunenshine RH, Wright MO, Maragakis LL, et al. MDR *Acinetobacter* infection mortality rate and length of hospitalization. Emerg Infect Dis 2007;13(1):97-103.
29. Peymani A, Nahaei MR, Farajnia S, et al. High prevalence of metallo-beta lactamase-*A.baumannii* in a teaching hospital in Tabriz-Iran. Japanese J Inf Dis 2011;64(1):69-71.
30. Principe L, D'Arezzo S, Capone APetrosillo N, Visca P. In vitro activity of tigecycline in combination with various antimicrobials against MDR *A.baumannii*. Annals Clin Microb Antimicrob 2009. URL: http:// annclinmicrob.com/content/8/1/18
31. Jin H, Xu XM, Mi ZH, Mou Y, Liu P. Drug resistent gene based genotyping for *A. baumannii* in tracing epidemiological events & for clinical treatment within nosocomial settings. Chinese Med J 2009;122(3):301-6.
32. Baran G, Erbay A, Bodur H, et al. Risk factors for nosocomial imipenem-resistent *A. baumannii* infections. Int J Infect Dis 2008;12(1):16-21.
33. Lee K, Yong D, Jeong SH, Chong Y. MDR *Acinetobacter* spp: increasingly problematic nosocomial pathogens. Yonsei Med J 2011;52(6):879-91.
34. Karuniawati A, Saharman YR, Lestari DC. Detection of Carbapenemase encoding genes in *Enterobacteria, P.aeruginosa* & *A.baumannii* isolated from patients at ICU Cipto Mangunkusumo 2011. Acta Med Indones 2013;45(2):101-6.