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RESEARCH

THE AUTOMATIC MICRODILUTION-BROTH IN SENSITIVITY TESTING OF ACINETOBACTER BAUMANNII ISOLATES

(Microdilution-Broth Otomatis Dalam Uji Kepekaan Isolat Acinetobacter Baumannii)

Dyah Artini, Osman Sianipar, Umi S Intansari

ABSTRAK

Acinetobacter baumannii (A.baumannii) merupakan bakteri Gram negatif, non-fermentatif dan non-motil yang seringkali menjadi penyebab infeksi pada manusia. Infeksi A.baumannii di Indonesia adalah sebanyak 25,8%. Belakangan ini telah dikembangkan metode microdilution-broth untuk uji kepekaan antimikrobia. Penelitian ini bertujuan untuk mengetahui ketidaktepatan metode microdilution-broth otomatis (Viteks2) dibandingkan dengan metode uji E (M.I.C.E.TM) secara mengukurnya. Penelitian potong lintang ini dilakukan terhadap 76 isolat klinik A.baumannii yang diperoleh dari pasien yang dirawat inap di RSUP Dr Sardjito Yogyakarta. Uji kepekaan meropenem dilakukan terhadap isolat klinik tersebut dengan menggunakan metode microdilution-broth otomatis (Viteks2) dan uji E (M.I.C.ETM). Patokan peka ≤4 ug/mL, intermediet 8 ug/mL dan resisten ≥ 16 ug/mL serta dilakukan perhitungan ketidaktepatan uji kepekaan meropenem metode microdilution-broth otomatis Viteks2. Isolat klinik A.baumannii sebagian besar diperoleh dari pasien rawat bukan gawat darurat 72,4% dan diikuti oleh yang berada di bangsal rawat yang gawat darurat dan poliklinik secara berturut-turut 21,1% dan 6,5%. Sumber sampel sebagian besar adalah nanah, darah dan air kemih berturut-turut 44,7%, 19,7% dan 14,5%. Metode microdilution-broth otomatis (Viteks2) menunjukkan 56,6% peka, 42,1% resisten dan 1,3% intermediet, sedangkan M.I.C.ETM menunjukkan 59,2% peka, 38,2% resisten dan 2,6% intermediet. Kesalahan kecil jika hasil M.I.C.ETM adalah Resisten (R)/Peka (P) dan Viteks2 adalah intermediet (I) atau M.I.C.ETM adalah I dan Viteks2 adalah R atau P. Kesalahan utama jika uji E M.I.C.ETM adalah P dan Viteks2 adalah R. Secara berturut-turut kesalahan kecil dan utama adalah 2,63% dan 2,63% (kurang dari 10%). Metode microdilution-broth otomatis (Viteks2) cukup tepat dalam menentukan uji kepekaan Meropenem terhadap A.baumannii.

Kata kunci: *Isolat klinik, A.baumannii, metode microdilution-broth otomatis (Viteks2), uji E*

ABSTRACT

Acinetobacter baumannii (A.baumannii) is a non-fermentive, non-motile Gram-negative bacteria which often causes infection in human. Infection caused by A.baumannii in Indonesia reaches approximately about 25.8%. Recently, an antimicrobial sensitivity testing known as automatic microdilution-broth method has been developed. The objective of the study was to determine the error rate of automatic microdilution-broth method (Viteks2) in comparison with Etest (M.I.C.Evaluator Strip or M.I.C.ETM). A Cross-sectional study was conducted in 76 clinical isolates of A.baumannii which were derived from in-patients at Dr. Sardjito Hospital Yogyakarta. The sensitivity testing of meropenem in those clinical isolates was performed by both automatic microdilution-broth method (Viteks2) and Etest (M.I.C.ETM) with MIC at ≤4 ug/mL, intermediate at 8 ug/mL and resistant at ≥16. The error rate of the automatic microdilution-broth method (Viteks2) was then calculated. The clinical isolates were mostly isolated from patients in non-intensive wards (72.4%), followed by patients in intensive ward (21.1%) and patients in out patients clinic (6.5%). The specimens were collected mostly from pus (44.7%), blood (19.7%) and urine (14.5%). The automatic microdilution-broth method (Viteks2) showed 56.6% sensitive, 42.1% resistant and 1.3% intermediate, whereas the M.I.C.ETM showed 59.2% sensitive, 38.2% resistant and 2.6% intermediate. Minor error was resulted when the result of M.I.C.ETM was Resistant (R)/Sensitive (S) and when the result of Viteks2 was Intermediate (I) or M.I.C.ETM resulted I and Viteks2 resulted R/S. Major error was resulted when the result of M.I.C.ETM was S and the result of Viteks2 was R. Both minor and major error were respectively 2.63% and 2.63% (less than 10%). The automatic microdilution-broth method (Viteks2) was quite accurate in determining the sensitivity testing of Meropenem to A.baumannii.

Key words: Clinical isolate A.baumannii, automatic microdilution-broth method, E test

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INTRODUCTION

Acinetobacter sp is a Gram-negative bacilli pleomorphic aerobic (similar to *Haemophilus influenzae* in Gram stain) commonly isolated from hospital and in-patients.[1]

Increased infection caused by *A.baumannii* has been occuring in the United States of America in the last decade. Data from The National Healthcare Safety Network show that *Acinetobacter baumannii* is the third bacteria with the most bacteria after *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while in the list of superiority of pathogens by the Infectious Diseases Society of America (IDSA), it occupies the fourth place.[2] The samples from bacteria have often been reported in Jakarta, Indonesia.[3] Gustawan[4] stated that among 2,729 clinical isolates taken from patients at children wards (ICU, PICU, NICU) in treatment and surgery related wards at Dr. Cipto Mangunkusomo Hospital, 47 blood isolates contained *A.baumannii*.[4]

Infection caused by *A.baumannii* is often found in the Intensive Care Unit (ICU) due to Ventilator-Associated Pneumonia (VAP), urinary tract infection and bacteremia.[5] Bacteria found in medical equipment cause special attention required in disinfection, wounds treatment and medical breathing apparatus.[6]

Also the infection caused by *A.baumannii* is getting more difficult to be treated due to resistant strains to many drugs emerging rapidly in health service section known as the hardest condition of resistance to control and treat. *A.baumannii* multidrug-resistance is rarely found in isolates taken from a community, but these bacteria are more frequently found in hospitals.[7] For almost 35 years, researchers observed the resistance of *A.baumannii* to antimicrobial-related drugs and frequently used drugs. The results showed that many *A.baumannii* strains were resistant to antimicrobials recently developed. As a result, the health worker is required to use antibiotics with a greater toxicity impact on patients. The availability of data related to infection caused by MDR *A.baumannii* will help health workers make the right decision in providing drugs and determine the course of disease.[8]

The number of death due to infection caused by TB *A.baumanii* is still high, approximately at 26% to 68%.[6] MDR *A.baumannii* can extend the length of hospitalization at ICU by six days and in hospital by 18 days (median).

Carbapenem (imipenem, meropenem or doripenem) is widely used as a mainstay for the treatment of severe infection caused by *A.baumannii* resistant strain. No wonder the strain of *Acinetobacter* resistant to carbapenem is rapidly found around the world, creating a challenge in treatment.[9] Research conducted by Mayasari[10] and Siregar[10] as well as by Kardana[11] showed that the resistance level of *A.baumannii* to meropenem was quite high.

Explanation about bacteria sensitivity to antibiotics may reduce morbidity and mortality, treatment costs and length of hospitalization if the updated description is received by health worker immediately and precisely.[12]

The methods used in antimicrobial sensitivity testing among others are disc diffusion and MIC based determination consisting of macrodilution and microdilution either in gelatine or broth which can be performed automatically and gradient diffusion.[13,14]

Manual procedures have been replaced with automatic system because of increased clinical specimens. Immediate results, ability to reproduce, ability to track result and great impact on working procedure also support the use of this system. However, some studies showed errors resulted from various automatic systems when some antimicrobial organisms were tested.[15] Genotype method cannot be applied in routine clinical test. Therefore, determining the sensitivity of particular antibiotics by Minimal Inhibitory Concentration (MIC) quantitatively which is useful in determining specific treatment is required.[15] Determination of antimicrobial treatment for resistant bacteria requires sensitivity testing with MIC level of antimicrobial concerned, so that they still can be used and associated with and has the lowest level of MIC although they are equally resistant.[16] Clinically, MIC is used not only to determine the amount of antibiotics to be administered to patient, but also the type of antibiotics, so that it is expected to decrease the possibility of resistance to certain antibiotics.[14] The criteria are acceptable (sensitive), intermediate, or resistant.[15] Some automatic systems providing data on bacteria sensitivity to antibiotics are offered. These systems generate results that have already been interpreted (vulnerable or resistant) or estimate the MIC between 3-10 hours after inoculation.[17] The method to determine MIC automatically is available in Vitek 2 based on microdilution-broth.[16] Problem arising due to the automatic system is a limited database.[17,18]

Measuring MIC using gradient endpoint method known as the E test is available as a product of AB Biodisk Solna, Sweden (Etest®) distributed by bioMerieux; M.I.C.E™ STRIP by Oxoid; and MIC Test Strip by Liofilchem. E test is a certain accurate, simple, easy and time-saving examination as a reference method so that dilution and broth for testing the sensitivity.[14,19] Arroyo *et al*[20] stated that E test was a simple and accurate standard examination method to examine *A.baumanni*. Examining using standard method is selected due to the stability of gradient antimicrobial on each strip in E test.[19] The basic form of E test is an in vitro method newly developed

in determining MIC, which can overcome some of the shortcomings of the procedures of disk diffusion and broth dilution, yet able to maintain the principle that benefits in generating MIC quantitatively.[21] Determining MIC with this method is to measure antimicrobial sensitivity quantitatively. However, due to the higher cost and limited availability in developing countries, the use of E test is still limited.[21]

In the interpretation and report of results of bacteria sensitivity testing, there may be inaccuracies and errors classified into minor error, major error and very major error. The inaccuracies can be seen by comparing the method of inspecting bacteria sensitivity testing with the reference.[24]

The inaccuracies in automated microdilution broth as well as in raw E test examination method are still found in various studies. The explanation of these inaccuracies is useful as a guide for health workers to administer antibiotics. The aim of this study was to determine inaccuracies in automated microdilution-broth method in sensitivity testing to meropenem in clinical isolates of *A.baumannii*.

METHODS

This study was an analytical observational study with cross sectional design involving 76 clinical isolates of *A.baumannii*. Samples collection and its examination using automatic microdilution-broth method known as Viteks2 and E test were carried out at the Sub-Section of Microbiology Parasitology and Infection, Pathology Section, Clinic of Faculty of Medicine, Gadjah Mada University/Clinical Laboratory of Dr. Sardjito Hospital Yogyakarta. The duration of sample collection was during September 2014-January 2015.

The medium and quality sterility test, quality control of automatic microdilution-broth and E test were carried out at initial stage of the research. Bacteria sensitivity testing to clinical isolates of *A.baumannii* was carried out to examine sensitivity to meropenem using automated microdilution-broth and E test. The results were classified as sensitive, intermediate or resistant, referring to criteria set by Clinical Laboratory Standard International (CLSI). Calculation of errors was carried to the two MIC results of both devices, consisting of minor error, major error and very major error. The minor error is concluded if the result of E test is Resistant (R)/ Sensitive (S) and if the result of Viteks2 is Intermediate (I) or the result of E test is I and the result of Viteks2 is R/S. The major error is concluded if the result of E test/P is sensitive and the result of Viteks2 is R. Certification regarding propriety of the research was issued by the Ethics Committee of the Medical and Health Research, Faculty of Medicine, Gadjah Mada University.

RESULTS AND DISCUSSION

The samples were largely pus, followed by blood, urine, sputum and others. Patients with clinical isolates were largely patients who were not in emergency care unit, followed by patients in the emergency care unit and the least was patients at the polyclinic.

In this study, the age range of patients was between 0 to 69 years, 30 patients were males, 36 patients were females, with the average age of 39.92 years. The results of this study were slightly different from the study conducted by Abbo *et al*[7] in which the number of male patients was greater than the number of female patients.[24] Another study involved more male patients with the average age of 66.2 years.[25] The samples in this study were largely taken from pus, different from the research conducted by Sieniawski[26] in which the samples of *A.baumanni* were largely taken from bronchial secretion. Noorhamdani[27] collected most samples from urine, followed by sputum, pus and blood.

Most of the patients in this study were from non-emergency care unit. Data taken from the Taiwan Surveillance of Antimicrobial Resistance Program from 2002 to 2010 showed that the majority of patients with infection caused by *A.baumannii* each year was not from the emergency care unit.[28]

The MIC of each clinical isolate of *A.baumannii* was calculated, using both automatic microdilution-broth Viteks2 and E test. The results of the calculation can be found in Figure 1 and 2 and Table 2.

Figure 1 showed that the number of samples with the MIC value of 16 was 33 samples, the MIC value of 8 and 4 was 1 sample each, the MIC value of 2 was four samples and the MIC value <2 was 37 samples.

Figure 2 showed that the number of samples with the MIC value of 32 was 19 samples, the MIC value of 16 was 11 samples, the MIC value of 8 was two samples and the MIC value of <4 was 44 samples.

The results showed that the MIC values of meropenem resulted from automatic microdilution-broth method and E test were nearly the same.

Table 1. Characteristics of clinical isolate of *A.baumannii*

Characteristics of subjects	N	%
Sample source		
Pus	34	44.73
Blood	15	19.74
Urine	11	14.47
Sputum	7	9.21
Others	9	11.84
Source of *A.baumannii* isolate		
Non-intensive care unit	55	72.36
Intensive care unit	16	29.1
Out patient clinic	5	6.57

This is in accordance with the research conducted by Kottahachchi[15] in 2012 in Sri Lanka. The results showed the same MIC value resulted from automated tool-broth microdilution and E test. The calculation resulted in the value of p=0.005; creating a significant difference between automated microdilution-broth method and E test.

The standard values that could not be accepted were >1.5% for very major error, 3% for major error and >10% for minor error.[29] The overall degree of error to be obtained in particular sensitivity testing in order to have an acceptable performance is a maximum of 10%.[15] The results shown in Table 3 were errors in Meropenem antibiotics in which the minor error was 2.63%, the major error was 2.63% and the very major error was 0%. These results were better than results of the study conducted by Kottahachchi[15] in SriLanka in 2012 in which the minor error was 8%. The results were acceptable because the overall degree of error was 5.26%, did not to exceed 10% as a condition for the performance of particular sensitivity testing to be accepted. The diversity of the medium was one of the weaknesses in this study because the researchers could not specifically observe the accuracy regarding the thickness of the medium used appropriately in growing the inoculum. Another factor was the possibility of contaminants from the air that could affect the results of this study.

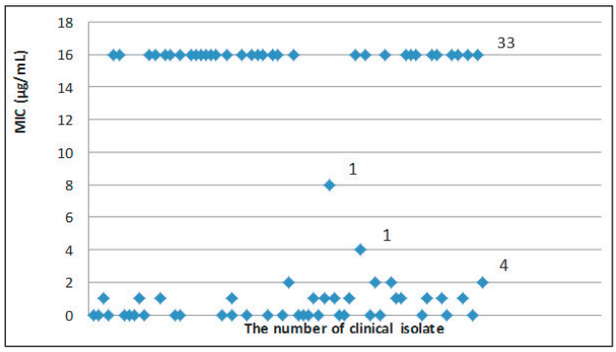

Figure 1. The number of clinical isolates of *A.baumannii* in accordance with the MIC value of meropenem using automatic microdilution-broth *(M.I.C.E^{TM})* method.

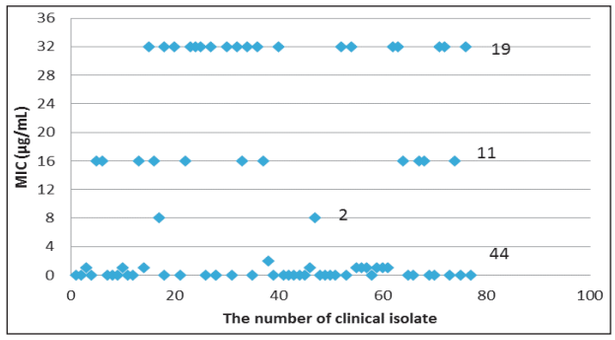

Figure 2. The number of clinical isolates of *A.baumannii* in accordance with the MIC value of meropenem using E test.

CONCLUSION

Automated microdilution-broth method had an inaccuracy of <10% in sensitivity testing to meropenem in clinical isolates of *Acinetobacter baumannii*. Thus, it can be used in sensitivity testing to meropenem antibiotics in clinical isolates of *Acinetobacter baumannii*.

Table 2. The MIC value of meropenem using automatic microdilution-broth and E test

Group	Automated microdilution-broth		E test	
	N	%	N	%
Sensitive (MIC≤4)	43	56.57	45	59.21
Intermediate (MIC8)	1	1.3	2	2.63
Resistant (MIC≥16)	32	42.1	29	38.15

Table 3. The analysis of inaccuracy in automated microdilution-broth and E test to Meropenem antibiotics

		E test			Total number
		Sensitive	Intermediate	Resistant	
Automated Microdilution-broth	Sensitive	42 (55.26%)	1 (1.31%)	0	43 (56.57%)
	Intermediate	1 (1.31%)	1 (1.31%)	0	2 (2.63%)
	Resistant	2 (2.63%)	0	29 (38.15%)	31 (40.78)
	Total number	45 (100%)	2 (2.63%)	29 (38.15)	76 (100%)

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