Investigation of *in-vitro* susceptibility of multidrug-resistant *Acinetobacter baumannii* strains isolated from clinical specimens to tigecycline

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Abstract

The management of infections due to *A. baumannii* is difficult because of rapidly developing resistance, however, tigecycline, a glycylcycline antimicrobial, is in use for several years. In the present study, it was aimed to determine the susceptibility rates of *A. baumannii* to tigecycline. A total of 90 *A. baumanni* isolates were tested using three methods such as disk diffusion, broth microdilution, and E-test. The MIC_{50} and MIC_{90} values and the MIC range were found as 2 µg/ml, 4 µg/ml, and 0.1-8 µg/ml by microdilution; and 2 µg/ml, 6 µg/ml, and 0.1-12 µg/ml by E-test, respectively. There were a few major errors as well as the minor rates were all high as between 35.7%-46.7%. The accuracy rates between the methods were low as 53.3% (48/90) between disk diffusion and E-test, 51.1% (46/90) between disk diffusion and microdilution, and 60.0% (54/90) between E-test and microdilution. In the ROC curve analysis, an inhibition zone diameter of susceptibility breakpoint of 21.5 mm had sensitivity between 68.8%-88.9%; specificity between 81.9%-87.9%; and accuracy between 80.0%-83.33%. An analysis based on EUCAST's non-species breakpoints, the MIC tests showed higher accuracy with a rate of 96.7%, however, performance of disk diffusion got worse as lower than 25%. In conclusion, we showed that the reliability of the methods even did not remain as high as the past. Our study presented that none of three methods revealed reliable results in determination of susceptibility of *A. baumanni* to tigecycline, so the clinical response should be followed up carefully in such cases.

KEY WORDS: tigecycline, *Acinetobacter*, Microdilution, Disk diffusion, E-test

INTRODUCTION

Acinetobacter baumannii is primarily associated with nosocomial infections particularly in patients with ventilator-associated pneumoniae and bacteremia and particularly in intensive care units (ICUs). Nosocomial infections due to this species is increasing in means of frequency with high rates of mortality particularly in critically ill patients [1, 2]. The management of infections due to *A. baumannii* is difficult because of rapidly developing resistance in this species. In addition, *A. baumannii* exhibits resistance to multi drug groups such as carbapenems, aminoglycosides and tetracyclines, meaning of reducing the therapeutic options. However tigecycline, a glycylcycline antimicrobial, is in use for several years with a mechanism of entering the bacterial cell through energy-dependent pathways or via passive diffusion, and then binding to the subunit 30S of the ribosomes, resulting the inhibition of protein syntesis of the microorganism. As a result tigecycline can escape from tetracycline efflux mechanism of the bacteria, meaning of causing slower and lower resistance in populations of *A. baumannii* [2, 3, 4]. Besides this, by the time tigecycline has been used in treatment of infections due to *A. baumannii*, resistance rates have been increasingly reported. In addition, the susceptibility breakpoints of inhibition zone diameter of tigecycline using disk diffusion test has changed over years. The question is whether the disk diffusion test still remains reliable [5, 6]. In the present study, it was aimed to determine the susceptibility rates of *A. baumannii* to tigecycline using three methods of disk diffusion, E-test and broth microdilution.

MATERIALS AND METHODS

Samples

In the present study, a total of 90 multidrug-resistant *A. baumannii* isolates were used for susceptibility tests. The isolates were obtained from cultures of respiratory tract specimens (37 isolates), blood (23 isolates), urine (17 isolates), and wound (13 isolates) all of which were processed in the microbiology laboratory of our hospital between

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October 2010 and May 2013. Because no molecular tests have been performed, we chose one isolate per patient in order to avoid to use any outbreak strains as we could. A total of 41 samples were collected from the inpatients of intensive care units of our hospital, 23 of them were from chest diseases clinics, 20 samples were from various surgery clinics, and the remaining six samples were from neurology. The identification of the isolates were done using BD Phoenix 100 (Becton Dickinson, USA) in species level.

Procedures

Three methods were used for determination of the susceptibility of the isolates to tigecycline. First of all, Kirby Bauer disk diffusion method was performed according to the recommendations of Clinical and Laboratory Standards Instittute (CLSI) [7]. Broth microdilution tests and E-test strips (Oxoid, United Kingdom) were used for determination of minimal inhibitor concentration (MIC) values of the antimicrobial as described [7]. For all the tests, isolates growth overnight and fresh as less than six hours manganese cation adjusted Mueller Hinton agar media were used. The MIC values and zone diameters were evaluated using both the previous criteria of British Society for Antimicrobial Chemotherapy (BSAC) for *A. baumannii* and European Committee on Antimicrobial Susceptibility Testing (EUCAST) for non-species testing [8, 9].

Statistical Analysis

A statistical analysis was performed using IBM SPSS Statistics Version 15 (SPSS Inc., Chicago, IL, USA). Continuous variables were tested for normality using the Shapiro-Wilk test. The breakpoints of disk diffusion zone diameters for predicting the susceptibility according to E-test and microdilution were analyzed using receiver operating characteristic (ROC) curve analysis. The sensitivity and specificity were presented when a significant cut-off value was observed. A p value of less than 0.05 was considered statistically significant. In addition, a major error was defined for the isolate that was found as susceptible by a method and as resistant by another method. In addition, minor error was defined for the isolate that was determined as intermediate by a method and as susceptible or resistant by another method. The accuracy rate was counted by division of true determination of the susceptibility according to each other of the methods by the total number.

RESULTS

The MIC_{50} and MIC_{90} values and the MIC range were found as 2 μg/ml, 4 μg/ml, and 0.1-8 μg/ml by microdilution; and 2 μg/ml, 6 μg/ml, and 0.1-12 μg/ml by E-test (Table 1). According to the methods the rates of susceptibility versus resistance were found as 16.7% (15/90) vs. 17.8% (16/90) by disk diffusion, 35.6% (32/90) vs. 25.6% (23/90) by microdilution, and 20.0% (18/90) vs. 38.9% (35/90) by E-test (Table 2). The accuracy rates between the methods were 53.3% (48/90) between disk diffusion and E-test, 51.1% (46/90) between disk diffusion and microdilution, and 60.0 (54/90) between E-test and micodilution. No major errors were found between disk diffusion and E-test. However, there were major errors in two isolates between disk diffusion and microdilution, and in four isolates between microdilution and E-test. The minor rates were all so high as between 35.7%-46.7% (Table 2). A ROC curve analysis was performed, and an inhibition zone diameter of 21.5 mm or more according to E-test susceptibility breakpoint had a sensitivity of 88.9%; specificity of 81.9%; positive predictive value of 55.2%; negative predictive value of 96.7%; and accuracy of 83.33% (Figure 1; AUC: 0.897 p<0.001 LB: 0.818 UB: 0.975) (when intermediate isolates are accepted as resistant). Another ROC curve analysis was made according to microdilution, and an inhibition zone diameter of 21.5 mm or more according to microdilution susceptibility breakpoint had a sensitiv-

TABLE 1. The Distribution of the MIC values according to the methods.

Methods	MIC_{50}	MIC_{90}	MIC range
Microdilution	2 μg/ml	4 μg/ml	0.1 μg/ml – 8 μg/ml
E-test	2 μg/ml	6 μg/ml	0.1 μg/ml – 12 μg/ml

MIC: Minimal inhibitory concentration.

TABLE 2. The analysis of susceptibility within the methods according to the previous BSAC criteria.

Disk Diffusion	E-test			Total	Analysis	
	S	I	R			
S	11	4	0	15	Major error	0/90
I	7	27	25	59	Minor error	42/90
R	0	6	10	16	Accuracy	48/90
Total	18	37	35	90		

Disk Diffusion	Microdilution			Total	Analysis	
	S	I	R			
S	13	1	1	15	Major error	2/90
I	18	26	15	59	Minor error	42/90
R	1	8	7	16	Accuracy	46/90
Total	32	35	23	90		

E-test	Microdilution			Total	Analysis	
	S	I	R			
S	17	0	1	18	Major error	4/90
I	12	20	5	37	Minor error	32/90
R	3	15	17	35	Accuracy	54/90
Total	32	35	23	90		

S: Susceptible, I: Intermediate, R: Resistant.

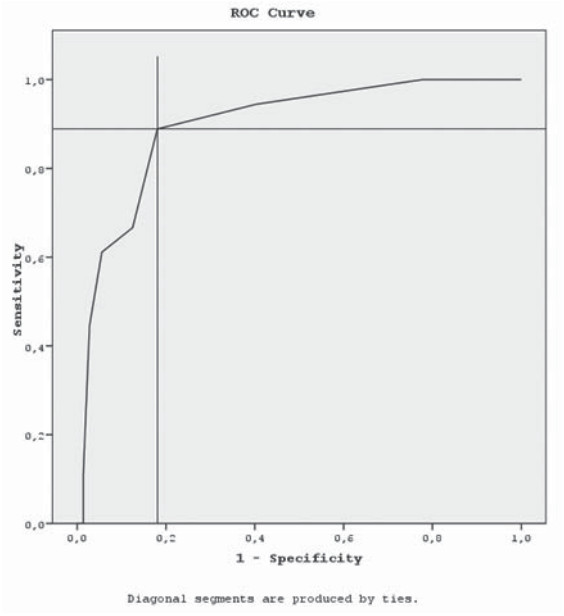

FIGURE 1. Inhibition zone diameter of 21.5 mm or more according to E-test susceptibility breakpoint had a sensitivity of 88.9%; specificity of 81.9%; positive predictive value of 55.2%; negative predictive value of 96.7%; and accuracy of 83.33% (AUC: 0.897 p<0.001 LB: 0.818 UB: 0.975) (When intermediate isolates are accepted as resistant).

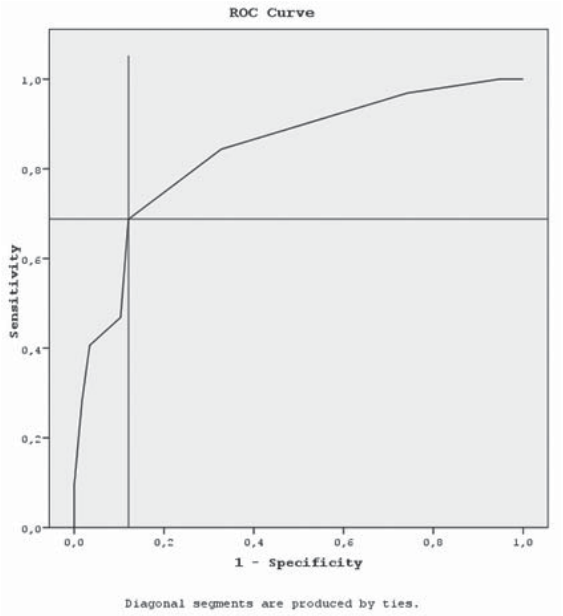

FIGURE 2. Inhibition zone diameter of 21.5 mm or more according to microdilution susceptibility breakpoint had a sensitivity of 68.8%; specificity of 87.9%; positive predictive value of 75.9%; negative predictive value of 83.6%; and accuracy of 80.00% (AUC: 0.838 p<0.001 LB: 0.750 UB: 0.926) (When intermediate isolates are accepted as resistant)

ity of 68.8%; specificity of 87.9%; positive predictive value of 75.9%; negative predictive value of 83.6%; and accuracy of 80.00% (Figure 2; AUC: 0.838 p<0.001 LB: 0.750 UB: 0.926) (When intermediate isolates are accepted as resistant). An analysis based on EUCAST's non-species breakpoints, the MIC tests showed higher accuracy with a rate of 96.7%, however, performance of disk diffusion got worse as lower than 25% (Table 3). In addition, no ROC curve analysis could be done for EUCAST criteria due to the low number of susceptible isolates.

TABLE 3. The analysis of susceptibility within the methods according to EUCAST criteria for non-species testing.

Disk Diffusion	E-test		Total	Analysis	
	S	R			
S	6	9	15	Major error	9/90
I	1	58	59	Minor error	59/90
R	0	16	16	Accuracy	22/90
Total	7	83	90		

Disk Diffusion	Microdilution		Total	Analysis	
	S	R			
S	7	8	15	Major error	8/90
I	1	58	59	Minor error	59/90
R	0	16	16	Accuracy	23/90
Total	8	82	90		

E-test	Microdilution		Total	Analysis	
	S	R			
S	6	1	7	Accuracy	87/90 (96.7%)
R	2	81	83		
Total	8	82	90		

S: Susceptible, I: Intermediate, R: Resistant.

DISCUSSION

Determination of susceptibility of the therapeutic agent that has been in a limited number of choise against *A. baumannii* is crucial particularly for critically ill inpatients. The method used on this topic has to be reliable and repeatable within the microbiology laboratories. Tigecycline has been used for treatment of this microorganism for several years as an efficient antimicrobial. However, the susceptibility rates have been reported to becoming changed [10, 11, 12, 13]. In the present study we showed that the reliability of the methods even did not remain as high as the past. We found that none of the three methods we used in the study was as accurate as we can trust. The MIC_{50} values for tigecycline were reported as 0.5 μg/ml by Souli et al. [14], Thamlikitkul et al. [15] and Seifert et al. [16]; 1 μg/ml by Draghi et al. [17] and Scheetz et al. [18]; as 2 μg/ml by Song et al. [19], Tan and Ng [20], Mezzatesta et al. [21], and Ratnam et al. [22]. Our MIC_{50} value seems to be concordant with these reports. However, the MIC_{90} values were found as 2 μg/ml by Sheetz et al. [18], Halstead et al. [23], Hohan et al. [24], Ratnam et al. [22], Mezzatesta et al. [21], and Draghi et al. [17]; and as 4 μg/ml by Tan and Ng [20], and Song et al. [19]. Our MIC_{90} value determined by

microdilution was also 4 μg/ml, but E-test showed a value of 6 μg/ml as the highest among these reports. These findings show the increase in MIC values over the years. Besides this, it was reported that some of the studies had revealed an overestimation of antimicrobial activity of tigecycline if more conservative previous BSAC breakpoints (≤1 μg/ml) was used compared with the one (≤2 μg/ml) that is widely accepted [1]. In the first years of use of tigecycline in infections caused by *A. baumannii*, the inhibition zone diameter breakpoints were recommended as 19 mm and 14 mm. However in the study by Kulah et al. [25] the recommendations for the breakpoints were 17 mm and 13 mm. Today, we seem to be so far away from these points. In the present study, we performed ROC analysis to determine a reliable zone diameter breakpoint according to either microdilution and E-test methods. However, a higher breakpoint of 21.5 mm in comparison to the past a few years' studies have still remained not so strong enough with its low sensitivities (Between 68.8%-88.9%) and specificities (Between 81.9-87.9). These findings showed that disk diffusion is not reliable any more for the determination of susceptibility to tigecycline. It was mentioned that the determination of the antimicrobial activity of tigecycline might vary with the use of different methods, and disk diffusion method could give lower susceptibility rates when compared to E-test or broth microdilution [1, 15, 22]. In the present study, we found so different susceptibility rates amongst the methods. The accuracy rates within the methods were low as between approximately 50%-55%. The disk diffusion test showed the worst performance on this topic. Besides this, the accuracy rate between microdilution and E-test methods, both of that are known to be more reliable methods, was the best with being not so high to be accepted, however, the highest major error rate was also observed between these two methods. Thamlikitkul et al. [15] also found different MIC_{50} and MIC_{90} values by microdilution and E-test methods as being four folds as 0.5 μg/ml vs. 2 μg/ml, and 1 μg/ml vs. 4 μg/ml, respectively. These results also showed the low reliability of all the three methods. The susceptibility testing criteria for tigecycline has changed in the last years. CLSI never suggested any breakpoints as well as EUCAST has now pulled back their criteria, and BSAC doesn't recommend any breakpoints with directing the reseachers to EUCAST's breakpoint for non-species testing to use [8, 9]. In this aspect, the number of susceptible isolates were decreased as lower than 10%, and the statistics has been biased. In this analysis, disk diffusion had accuracy rates below 25%. However, microdilution and E-test methods showed greater concordance as 96.7%. These aspects support that there has been an invalidation on susceptibility testing of tigecycline against *A. baumanni*.

CONCLUSION

Our study presented that neither disk diffusion test nor microdilution and E-test methods have lack of reliability in determination of susceptibility of *A. baumanni* to tigecycline. All the three methods revealed inaccordant results to each other. In accord with the recommendations of BSAC, EUCAST, and CLSI, we consider that none of the susceptibility tests and interpretive criteria can give accurate results, so the clinical response should be followed up carefully in cases of giving tigecycline to a patient with an infection caused by *A. baumannii*.

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DECLARATION OF INTEREST

The authors declare no conflict of interest.

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