In Vitro Activity of Tigecycline against Clinical Isolates of Multidrug-Resistant *Acinetobacter baumannii* in Siriraj Hospital, Thailand

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In vitro activity of tigecycline against 148 strains of Acinetobacter baumannii isolated from different patients hospitalized at Siriraj Hospital, Bangkok, Thailand during 2002 to 2005 was conducted. These isolates were resistant to beta-lactams, aminoglycosides and fluoroquinolones. In vitro susceptibilities were determined by Kirby-Bauer disk diffusion, E-test and broth microdilution methods. The MIC_{50} and MIC_{90} values of tigecycline against A. baumannii determined by the broth microdilution method were 0.5 and 1 mg/L respectively. The MICs of tigecycline determined by E-test were 4-fold higher than those from the broth microdilution method. An inhibition zone of ≥13 mm was well correlated with a tigecycline MIC of ≤ 2 mg/L and had a sensitivity of 99% and a specificity of 100%. The study results indicated that 97.3% of MDR A. baumannii strains isolated from the patients hospitalized at Siriraj Hospital were susceptible to tigecycline. Tigecycline may prove to be an important antibiotic for treatment of multidrug-resistant A. baumannii infections in Thailand in the near future.

Keywords: *Tigecycline, Acinetobacter baumannii*

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Acinetobacter baumannii has emerged as a worldwide problem in causing infections in hospitalized patients[1-3]. *A. baumannii* is one of the most common causative pathogens in nosocomial pneumonia, bacteraemia, urinary tract infections, and skin and soft tissue infections, and the mortality associated with these infections is high. The incidence of infections caused by multidrug-resistant (MDR) pathogens, particularly *Acinetobacter baumannii* and *Pseudomonas aeruginosa,* in Thailand has dramatically increased[4]. A prospective study of 208 clinical isolates of *A. baumannii* recovered from patients in Siriraj Hospital from January to December 2002 revealed that 86 strains (41.3%) were isolated from infected patients and the remaining 58.7% were colonizers[5]. In this study, 57% of *A. baumannii* isolates were resistant to all antimicrobial agents available in Thailand including beta-lactams, aminoglycosides and fluoroquinolones, and the overall mortality rate of the patients infected with pandrug-resistant *A. baumannii* was 79%[5]. The study of 104 clinical isolates of *A. baumannii* from 100 hospitalized patients at Maharaj Nakorn Chiang Mai Hospital, Thailand also observed that 46% of the isolates were pandrug-resistant and the overall mortality was 52%[6]. The only available antibiotic effective for treating infections caused by *A. baumannii* resistant to all beta-lactams,aminoglycosides and fluoroquinolones is colistin[7], hence a search for new agents effective against MDR *A. baumannii* is needed.

Tigecycline is a glycylcycline antibiotic that shows promising activity against a wide range of organisms including multi-drug resistant gram positive cocci and gram negative bacilli[8]. The objective of the study was to determine in vitro activity of tigecycline against clinical isolates of MDR *A. baumannii* in

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Material and Method

One hundred and forty-eight strains of *A. baumannii* isolated from different infected patients hospitalized at Siriraj Hospital, Bangkok, Thailand during 2002 to 2005 were included. These isolates were resistant to all beta-lactams, aminoglycosides, and fluoroquinolones. In vitro susceptibilities of MDR *A. baumannii* to tigecycline were determined by Kirby-Bauer disk diffusion, E-test, and broth microdilution methods. Paper disc containing tigecycline 15 μg per disk (Becton Dickinson, USA), E-test strips (AB BIODISK, Sweden) and gram negative MicroScan MIC panels (Dade Behring Inc., USA) were provided by Wyeth Research. The methodology for susceptibility testing was done by direct colony suspension according to guidelines suggested by CLSI [9]. Quality control was performed by testing the susceptibility of *E. coli* ATCC 25922 as recommended by Wyeth Research.

Results

A distribution of inhibition zone diameters of tigecycline against *A. baumannii* is shown in Table 1. The MIC_{50} and MIC_{90} values of tigecycline against *A. baumannii* determined by E-test were 2 and 4 mg/L respectively. The MIC_{50} and MIC_{90} values of tigecycline against *A. baumannii* determined by the broth microdilution method were 0.5 and 1 mg/L respectively. There was a significant correlation between inhibition zone diameters and MICs determined by the broth microdilution method ($p<0.001$, $r = -0.8$), and between MICs of tigecycline determined by E-test and MICs determined by the broth microdilution method ($p<0.001$, $r=0.9$). The accuracy of the inhibition zone diameter of ≥13 mm in predicting susceptibility of *A.baumannii* to tigecycline is shown in Table 2. If the MIC of tigecycline at ≤2 mg/L was considered as a breakpoint for tigecycline susceptibility, the inhibition zone diameter of ≥13 mm had a sensitivity of 99% and a specificity of 100% in predicting the susceptibility of *A.baumannii* to tigecycline and 97.3% of MDR *A.baumannii* were susceptible to tigecycline.

Discussion

The previous studies on the in vitro activity of tigecycline against *A.baumannii* by the broth microdilution method revealed that the MIC_{50} and the MIC_{90} for tigecycline were 0.5-1 and 2 mg/L respectively, and more than 90% of these isolates had MICs ≤2 mg/L and were considered susceptible to tigecycline[10, 11]. Carbapenem-resistant *A. baumannii* isolates were still susceptible to tigecycline with comparable MICs to the aforementioned values[12, 13]. However, in vitro activity of tigecycline against *A. baumannii* by the agar dilution method observed that the MIC_{50} and the MIC_{90} for tigecycline against *A.baumannii* were 8 and 8 mg/L respectively[14]. These findings implied that the different methods of in vitro susceptibility testing of tigecycline against *A. baumannii* might yield different results. The breakpoints for the inhibition zone diameter and MIC of tigecycline against *A.baumannii* are not available. The US FDA-approved breakpoints of tigecycline against Enterobacteriaceae to be used by the local laboratory were inhibition zone diameter ≥19 mm and a MIC≤2 mg/L[9]. The previous studies on in vitro activity of tigecycline against *A. baumannii* used such a MIC breakpoint[10-14]. It is not known if the testing methods used in general microbiology laboratories, disk diffusion and E-tests, are accurate in predicting the MICs of tigecycline against *A. baumannii*.

The MIC_{50} and the MIC_{90} of tigecycline against *A. baumannii* determined by the broth microdilution method observed in our study were similar to those reported in the literature[10-13]and 97.3% of MDR *A. baumannii* isolated from the hospitalized patients at Siriraj Hospital were susceptible to tigecycline. However, our findings indicated that there was a discrepancy in the susceptibility results of tigecycline against *A. baumannii* for the different

Table 1. Distribution of the inhibition zone diameter of tigecycline against 148 isolates of MDR *A. baumannii*

Inhibition Zone Diameter (mm)	Number of Isolates (%)
11	1 (0.7)
12	4 (2.7)
13	4 (2.7)
15	8 (5.4)
16	11 (7.4)
17	20 (13.5)
18	34 (23.0)
19	21 (14.2)
20	17 (11.5)
21	16 (10.8)
22	8 (5.4)
23	3 (2.0)
26	1 (0.7)

Table 2. Accuracy of the inhibition zone diameter of ≥13 mm in predicting the susceptibility of *A.baumannii* to tigecycline

	MIC (MicroScan) ≤ 2 mg/L	MIC (MicroScan) > 2 mg/L
Inhibition Zone Diameter ≥13 mm	143	0
Inhibition Zone Diameter <13 mm	1	4

methods of testing. The MICs determined by E-test were usually 4-fold higher than those determined by the broth microdilution method and E-test might not be an accurate method for in vitro susceptibility testing of tigecycline against *A. baumannii*. Moreover, our study also observed that the US FDA-approved breakpoint of tigecycline against Enterobacteriaceae, to be used by the local laboratory, of an inhibition zone diameter ≥19 mm, was not applicable to tigecycline against *A. baumannii*. The breakpoint for an inhibition zone diameter ≥13 mm was more accurate in predicting susceptibility of *A.baumannii* to tigecycline with a sensitivity of 99% and a specificity of 100%. Our findings of good in vitro activity of tigecycline against MDR *A. baumannii* warrant a clinical study to prove its efficacy and to determine whether such proposed breakpoint and testing methods are valid.

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การทดสอบฤทธิ์ของ tigecycline ต่อ Acinetobacter baumannii ที่ดื้อยาต้านจุลชีพหลายขนานที่แยกได้จากผู้ป่วยในโรงพยาบาลศิริราช

สุรภี เทียนกริม, ชาญวิทย์ ตรีพุทธรัตน์, วิษณุ ธรรมลิขิตกุล

ผู้วิจัยได้ทดสอบฤทธิ์ของ tigecycline ต่อ Acinetobacter baumannii ที่ดื้อยาต้านจุลชีพหลายขนานที่แยกได้จากผู้ป่วยของโรงพยาบาลศิริราชระหว่าง พ.ศ. 2545 ถึง พ.ศ. 2548 จำนวน 148 สายพันธุ์ด้วยวิธี disk diffusion และวัด minimum inhibitory concentration (MIC) ด้วย E-test และ broth microdilution พบว่า 1) ค่า MIC_{50} และ MIC_{90} ของ tigecycline ต่อ A. baumannii ที่ดื้อยาต้านจุลชีพหลายขนานที่ทดสอบด้วยวิธี broth microdilution เท่ากับ 0.5 มก./ล. และ 1 มก./ล. ตามลำดับ 2) ค่า MIC_{50} และ MIC_{90} ของ tigecycline ต่อ A. baumannii ที่ดื้อยาต้านจุลชีพหลายขนานที่ทดสอบด้วยวิธี E-test มีค่ามากกว่าค่า MIC ที่ได้จากวิธี broth microdilution ประมาณ 4 เท่า 3) เส้นผ่านศูนย์กลางของ inhibition zone ของ tigecycline $\geq$13 มม.เป็นค่าที่เหมาะสม สำหรับพิจารณาความไวของ A. baumannii ต่อ tigecycline หากใช้เกณฑ์ MIC$\leq$2 มก./ล. ในการระบุว่า A. baumannii ไวต่อ tigecycline โดยมีความไวร้อยละ 99 และความจำเพาะร้อยละ 100, 4) Acinetobacter baumannii ที่ดื้อยาต้านจุลชีพหลายขนานร้อยละ 97.3 ไวต่อ tigecycline ดังนั้น tigecycline น่าจะมีประโยชน์ในการรักษาโรคติดเชื้อ Acinetobacter baumannii ที่ดื้อยาต้านจุลชีพหลายขนานในประเทศไทย