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Tigecycline Susceptibility in Multidrug Resistant *Acinetobacter* Isolates from Turkey

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Abstract

The present study aimed to evaluate antimicrobial activity of tigecycline against 84 multidrug resistant (MDR) *Acinetobacter* spp. strains by disc diffusion and E-test methods. The results of disc diffusion test were compared according to two different interpretation ways. In addition, E-test results and the disc diffusion results that interpreted by both the methods were checked for compatibility. According to the disc diffusion test, 3 strains (3.57%) were found resistant to tigecycline when considering breakpoints suggested by Food and Drug Administration (FDA). On the other hand, none of the strains was found resistant to the evaluation criteria recommended by Jones *et al.* (2007). Considering E-test results of tigecycline, MIC_{50} and MIC_{90} values of tigecycline for *Acinetobacter* spp. were 0.75 and 1 mg/l, respectively. Based on FDA defined breakpoints for *Enterobacteriaceae*, any resistant isolate was detected. In conclusion, although there are some differences in the results, tigecycline was found quite effective on *Acinetobacter* spp. isolates with reference to the both disc diffusion and the E-test methods.

Key words: *Acinetobacter*, antibiotic resistance, tigecycline

Introduction

Acinetobacter spp. is important opportunistic pathogen in nosocomial infections, which cause a wide range of clinical complications, such as pneumonia, septicemia and meningitis, especially in immunocompromised patients and intensive care units (ICUs). In recent years, new antibacterial agents are needed for the treatment of infections caused by multidrug-resistant (MDR) *Acinetobacter* spp., including broad-spectrum beta (β)-lactams, aminoglycosides, and fluoroquinolones (Falagas *et al.*, 2008; Manchanda *et al.*, 2010; Neonakis *et al.*, 2011). Tigecycline was recently approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency for the treatment of complicated skin and intra-abdominal infections. Tigecycline, the 9-tert-butyl-glycylamido derivative of minocycline, exhibits a broad-spectrum of activity against numerous pathogens, including *Acinetobacter* spp.. Like the tetracyclines, tigecycline binds to the 30S subunit of bacterial ribosomes and inhibits protein synthesis by preventing the incorporation of amino acid residues into elongating peptide chains (Fraise, 2006; Neonakis *et al.*, 2011; Peterson, 2008).

However, many researches indicated that there was a discrepancy in the susceptibility results of tigecycline against *Acinetobacter* spp. among different methods of testing such as broth microdilution, E-test, disc diffusion, and automated systems. Reference standard, broth microdilution testing serves as the method of comparison for the development and evaluation of alternative susceptibility testing methodologies. Recently, an E-test has been developed for the susceptibility testing of tigecycline. However, defined susceptibility breakpoints have not been declared thus far for *A. baumannii* in the latest issues of the Clinical and Laboratory Standarts Institute (CLSI) because of insufficient data about clinical usage of tigecycline (Liu *et al.*, 2010; Neonakis *et al.*, 2011; Shakoor *et al.*, 2011). The unavailability of standard breakpoints of tigecycline leads to mistakes in categorization of MIC values and consequently gives rise to careless use of this antibiotic (Shakoor *et al.*, 2011).

The first aim of the present study was to investigate the antimicrobial activity of tigecycline by disc diffusion

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method and the E-test for 84 clinical MDR *Acinetobacter* sp., and the second one was to compare the susceptibility assessment methods.

Experimental

Material and Methods

Bacteria. Between December 2009 and December 2010, 84 MDR *Acinetobacter* spp. isolates were collected from various clinical specimens at İzmir Katip Çelebi University, Atatürk Training and Research Hospital, Medical Microbiology Laboratory, Turkey. From the total 84 specimens obtained, 67 (80%) were from ICUs. The isolates were identified and antimicrobial susceptibilities were determined by BD Phoenix System. MDR *Acinetobacter* spp. were defined as the isolates resistant to at least three classes of antimicrobial agents. The isolates were stored at –80°C, in the Brain Heart Infusion broth (Oxoid) supplemented with 10% glycerin.

Disc diffusion method. *In vitro* susceptibility of *Acinetobacter* spp. against tigecycline was determined by Kirby-Bauer disc diffusion method according to the CLSI guidelines, by using 15 μg tigecycline discs (Becton Dickinson, USA) (CLSI). The results were evaluated by using disc diffusion breakpoints for *Enterobacteriaceae* proposed by FDA (susceptible ≥ 19 mm and resistant ≤ 14 mm) and by Jones *et al.* (2007) (susceptible ≥ 16 mm and resistant ≤ 12 mm). *Escherichia coli* ATCC 25922 was used as control strain.

E-test method. E-test Tigecycline gradient strips (AB Biodisc, Sweden; 0.016–256 μg/ml) were used according to CLSI guidelines and the MIC values were interpreted according to FDA defined breakpoints for *Enterobacteriaceae* (susceptible ≤ 2 mg/l; intermediate 4 mg/l; resistant ≥ 8 mg/l) were applied in this study. MICs were read at 100% inhibition of growth. *E. coli* ATCC 25922 was used as the control strain.

Statistical analysis. Statistical analysis was performed using Minitab statistical software (Minitab Release 16©, State College, PA). For comparison of the evaluation criteria and antibiotic susceptibility tests results, Z test was employed. In all tests, differences were considered significant when $p < 0.05$.

Results

This study showed that 3 *Acinetobacter* spp. strains (3.57%) were resistant according to a disc diffusion method when considering breakpoints suggested by FDA. None of the strains was found resistant in the disc diffusion results according to Jones' criteria. Similarly, E-test method results showed no resistance in the *Acinetobacter* spp. strains. On the other hand, the susceptibility rate detected by the E-test method was statistically higher than the disc diffusion method according to both interpretation criteria ($p < 0.05$) (Table I).

The tigecycline MIC range was found as 0.032–3 mg/l by E-test method. MIC_{50} and MIC_{90} values of tigecycline for *Acinetobacter* spp. were 0.75 and 1 mg/l, respectively (Table II).

Table I
Comparing the tigecycline susceptibility of 84 *Acinetobacter* spp. isolates

Methods	Evaluation criteria	S n (%)	I n (%)	R n (%)
Disc Diffusion	FDA[a]	46 (54.76)	35 (41.67)	3 (3.57)
	Jones criteria[b]	69 (82.14)	15 (17.86)	–
E-test	FDA[c]	83 (98.81)	1 (1.19)	–

S: Susceptible, I: Intermediate, R: Resistant;
[a] FDA criteria for disc diffusion method: S ≥ 19 mm, R ≤ 14 mm
[b] Jones criteria for disc diffusion method: S ≥ 16 mm, R ≤ 12 mm
[c] FDA criteria for E-test method: S ≥ 2 μg/ml, R ≤ 8 μg/ml

Discussion

Recently, some researches on *in vitro* activity of tigecycline against *Acinetobacter* showed a variability depending on the methodology used to determine susceptibility. For example, microdilution testing methodologies can show potent *in vitro* activity for tigecycline against MDR *Acinetobacter* spp., on the other hand the E-test can indicate high tigecycline resistance among clinical isolates (Kulah *et al.*, 2009; Shakoor *et al.*, 2011; Wang and Dowzicky, 2010). In this study, all the *Acinetobacter* sp. isolates were found to be susceptible to tigecycline although there were some differences in the results of the E-test and disc diffusion assays. Besides, E-test susceptibility results were supported by disc diffusion results when the recommendations by Jones *et al.*

Table II
MIC results of 84 *Acinetobacter* spp. isolates according to the E-test method

	MIC values (μg/ml)												
	0.032	0.064	0.094	0.125	0.19	0.25	0.38	0.5	**0.75**	**1**	1.5	2	3
n	1	5	6	9	6	4	2	8	23	13	3	3	1
(%)	(1.19)	(6.0)	(7.2)	(10.8)	(7.2)	(4.8)	(2.4)	(9.5)	(27.4)	(15.5)	(3.6)	(3.6)	(1.2)

n: Number of strain, MIC_{50}: 0.75 μg/ml, MIC_{90}: 1μg/ml

(2007) were considered ($p < 0.05$), and the MICs of the isolates did not exceed 3 mg/l.

Wang and Dowzicky (2010) found low MIC_{90} values (≤ 2 mg/l) for tigecycline against *Acinetobacter* spp. isolates from blood samples, as similar studies published before. They also pointed out the deficiency of suggested breakpoints for tigecycline against *Acinetobacter* spp., thus comparing susceptibility studies based on different guides leads to confusing or even misleading results. When considereding the FDA breakpoint for *Enterobacteriaceae*, tigecycline inhibited at least 90.0% of isolates from all countries (Mendes *et al*., 2010).

In the east part of Turkey, of 71 *A. baumannii* strains studied, 2 strains (3%) were resistant, 35 strains (49%) moderately susceptible, and 34 strains (48%) susceptible against tigecycline according to the disk diffusion breakpoints proposed by FDA for *Enterobacteriaceae* and 1 strain (1%) was resistant, 1 strain (1%) moderately susceptible, and 69 strains (97%) susceptible when considered the breakpoints according to Jones *et al*. Thus, it was asserted that the use of FDA criteria for tigecycline against *Acinetobacter* spp. was inaccurate, and also breakpoints suggested by Jones *et al*. should be supported by further investigations (Gülhan *et al*., 2009).

Bolmstrom *et al*. (2007) showed that the tigecycline E-test gradient method was as accurate as the reference methods. In addition, the error rates were very low. Hope *et al*. (2007) determined that tigecycline E-tests were shown to have good correlation with agar dilution MICs. However, Thamlikitkul *et al*. (2007) indicated that there was a discrepancy in the susceptibility results of tigecycline against *Acinetobacter* spp. among different methods of testing. The MICs determined by the E-test were usually four-fold higher than those determined by the broth microdilution method. Similarly, Pillar *et al*. (2008) observed a four-fold increase in MIC_{90} value among tested *A. baumannii* by E-test relative to broth microdilution test and noted a difference between the two testing methodologies. Liu *et al*. (2010) compared the results of E-test and broth microdilution method for tigecycline susceptibility testing of 393 *A. baumannii* isolates collected from 19 hospitals in Taiwan. E-test results showed an agreement in 76.6% of the strains when compared with the broth microdilution method. According to the results they declared that the E-test is not ideal as a substitute for broth microdilution testing in determining the MICs of tigecycline against *A. baumannii* isolates. Zarate *et al*. (2010) assayed in parallel by the broth microdilution, agar dilution, and disc diffusion method in 60 MDR *Acinetobacter* spp. isolates obtained from hospitalized patients at two teaching hospitals in Argentina. A comparative analysis between methods by scattergram correlation and analysis of MICs and diameter zones around the disk was performed. They found a positive lineal correlation between the methodologies. Using the FDA *Enterobacteriaceae* susceptibility breakpoint for tigecycline, an acceptable minor error rate was observed by agar dilution and broth microdilution, but an unacceptable error by the disc diffusion method. In another study from Pakistan (Shakoor *et al*., 2010), *in vitro* activity of tigecycline against 100 *Acinetobacter* spp. were determined by E-test and the MICs were interpreted according to both the BSAC and FDA breakpoints. Their data has changed significantly from 94% sensitive to 79% non-susceptible (intermediate or resistant), thus the authors underlined the importance of requirement universally compliant breakpoints for tigecycline against *Acinetobacter* spp.

Conclusions

Management of *Acinetobacter* spp. infections is difficult due to the emergence of isolates with multiple-drug resistance. Thus, it is necessary to evaluate new molecules that are potentially useful against *Acinetobacter* spp. Tigecycline is seems to be a good choice for succeed in therapy. It is also an important to monitor the increase of the resistance in the microorganisms during the usage of tigecycline for treatment. The development and validation of reliable methods for antimicrobial susceptibility testing and MIC determinations of tigecycline are critical to clinical practice as well as for ongoing surveillance programs.

In many countries, agar dilution or broth microdilution method is recommended, because the tigecycline microdilution panel is still difficult to obtain on a large scale. The E-test strip can be set up as easily as a disc diffusion test by most clinical laboratories without the need for specialized equipment. The disc diffusion data should be supported by broth microdilution tests and further studies should be conducted to minimize false-susceptible errors. It is also important to decide the evaluation criteria to determine the antibiotic susceptibility properly. Interpretive breakpoints for susceptibility reporting by clinical microbiology laboratories were previously set for an antimicrobial agent with no consideration of bacterial species differences. In recent years such differences have been appreciated and species-related interpretive breakpoints are issued more frequently. Moreover, further studies are needed to define the most adequate methods for testing tigecycline susceptibility in *Acinetobacter* sp.

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