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, MIC50 and MIC90 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 Standards 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
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 Izmir 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. The isolates 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. MIC₉₀ and MIC₅₀ values of tigecycline for Acinetobacter spp. were 0.75 and 1 mg/l, respectively (Table II).

**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. were considered.
breakpoints proposed by FDA 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 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 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 spp.

Literature


