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In vitro activities of tigecycline against *Brucella* spp. in an endemic area

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Human brucellosis remains a common zoonotic disease worldwide; it has been estimated to cause 500,000 new cases annually. In Saudi Arabia, brucellosis is endemic in different parts of the country due to consumption of unpasteurized milk. In this study, we investigated *in vitro* activity of tigecycline (TIG) against *Brucella* spp. isolated at Microbiology Laboratory, King AbdulAziz Medical City over the last 15 years. A total of 704 *Brucella* species isolates tested against TIG using E-test and MIC were measured. Of the 704 *Brucella* strain sub cultured, there were 444 isolates (63.07%) with an MIC equal or below 0.125 µg/ml, and the remaining 260 (36.93%) has an MIC between (0.190-2.0 µg/ml). The results of this *in vitro* study suggest that tigecycline has a promising future to be used as a therapeutic alternative for brucellosis. These observations need to be supported with clinical trials. This is the first study that analyzes a large number of *Brucella* isolates in our region.

Key words: Tigecycline, *Brucella*, Saudi Arabia.

INTRODUCTION

Human brucellosis remains a common zoonotic disease worldwide; it has been estimated to cause 500,000 new cases annually (Pappas et al., 2006). Brucellosis caused by Gram-negative bacteria, *Brucella* spp. can be transmitted to humans through direct contact with infected animals, consumption of dairy products or inhalation of aerosols (Young et al., 2005).

Brucellosis represents serious consequences for public health; as it is associated with long treatment, slow recovery and possible serious complications in different body systems mainly the musculoskeletal and nervous system as well as a major issue with recurrent relapses (Young et al., 2005). Although brucellosis has been eradicated in many northern European countries, in Australia, New Zealand, and Canada due to the implementation of national surveillance program and vaccination of livestock, it is still endemic in the Mediterranean basin, Middle East, Southwest Asia and parts of Latin America (Pappas et al., 2006; Black et al., 2004). In Saudi Arabia, brucellosis is endemic in different part of the country (Al-Tawfiq and Abukhamsin, 2009; Elbeltagy, 2001; Fallatah et al., 2005). The genus *Brucella* is an intracellular bacterial pathogen that infects host macrophage cells. Consequently, specialized agents that are able to penetrate the macrophages and function within their cytoplasm are required for the treatment of brucellosis (Young et al., 2005). Therefore, a limited number of antibiotics are effective against these

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organisms. In 1986, the WHO has released recommendations for use of doxycycline, combined with either rifampin or streptomycin for treating human brucellosis (Joint Food and Agriculture Organization/World Health Organization, 1986). Although this recommendation is still in function and *Brucella* isolates are generally considered susceptible to the recommended WHO antibiotics, sporadic cases of a kind of antibiotic resistance have been reported (Baykam et al., 2004; Lopez-Merino et al., 2004). Up until 2006, *in vitro* antimicrobial susceptibility testing of *Brucella* spp. is not standardized and not generally recommended due to risk of laboratory-acquired infection and requirement of biological safety level precautions, so there are few studies on this issue in the literature (Baykam et al., 2004; Lopez-Merino et al., 2004; Bodur et al., 2003; Köse et al., 2005; Dizbay et al., 2007; Kilic et al., 2008; Garcia-Rodriguez et al., 1991; Qadriet al., 1995; Kilic et al., 2008).

In vitro susceptibilities of antibiotic susceptibility testing (AST) may change over time and from one geographical region to another (De Rautlin et al., 1986; Kinsara et al., 1999).

Side effects of drug combinations along with the high incidence of relapses and therapeutic failures, have led to the investigation of new drugs to treat the disease.

Tigecycline (TIG), a member of a new class of antimicrobials, the glycylcyclines, may serve as alternative drug choices (Dizbay et al., 2007; Kilic et al., 2008; Garcia-Rodriguez et al., 1991; Qadri et al., 1995; Kilic et al., 2008; De Rautlin et al., 1986).

The aim of this study was to investigate *in vitro* activity of tigecycline (TIG) against *Brucella* spp. isolated at Microbiology Laboratory, King AbdulAziz Medical City over the last 15 years.

MATERIALS AND METHODS

Bacterial strains

A total of 760 samples were tested; of these, 471 *Brucella* isolates were collected retrospectively between 1997 - December 2008 and 289 tested prospectively from January 2009 till December 2012. All samples are from blood and sterile body fluid cultures of patients with acute brucellosis who were at King AbdulAziz Medical City, Riyadh, Saudi Arabia (KAMC-R).

Identification methods

Identification of species was made on the basis of Gram staining reaction, colonial morphology, CO_2 requirements for growth, production of urease and H_2S (Alton et al., 1988; Bayram et al., 2011). The strains were stored in glycerol at -80°C and subcultured twice prior to performing of AST.

Antimicrobial susceptibility testing

Minimum inhibitory concentration (MIC) of tigecycline (TIG) were determined by E-test (Biomerieux, Sweden) method on Mueller-Hinton agar (Oxoid, Basingstoke, UK) supplemented with 5% sheep blood and interpreted after 48 h of incubation at ambient air. Mueller-Hinton agar supplemented with 5% sheep's blood was inoculated with suspensions of the test organism equivalent 0.5 McFarland turbidity, and E-test strips were applied onto culture plates. The plates were incubated in ambient air at 35°C and read after 48 h. The MIC was interpreted as the value at which the inhibition zone intercepted the scale on the E-test strip. MIC_{50} and MIC_{90} levels were defined as the lowest concentration of the antibiotic at which 50 and 90% of the isolates were inhibited, respectively. We used *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 as the quality control strain for antimicrobial susceptibility testing (Bayram et al., 2011).

RESULTS

A total of 760 *Brucella* strain were subcultured, out of the 760, 56 isolates were excluded because they fail to grow (8 isolates), or were not tested against TIG (48 isolates). The remaining 704 isolates were tested against TIG using E-test, the MIC were determined for each isolates. MIC range (0.064-0.125 µg/ml): MIC_{50} (0.064 µg/ml) and MIC_{90} (0.094 µg/ml). Out of 704 isolates, 444 isolates (63.07%) has an MIC equal or below 0.125 µg/ml, and the remaining 260 (36.93%) has an MIC between (0.190-2.0 µg/ml) (Figure 1).

Of the 444 presumed susceptible strains, there were 156 isolates (35.14%) with MIC below 0.064 µg/ml and 291 (65.54%) has MIC between 0.064-0.125 µg/ml.

Of the 704 isolates tested, there were 612 (86.93%) from blood culture samples, 27 from synovial fluid, 12 from peritoneal fluids, six from cerebrospinal fluid and the remaining from different sources.

DISCUSSION

Brucellosis is an endemic zoonotic infectious disease in Saudi Arabia. To date, there is no optimum antibiotic therapy for brucellosis due to relatively high rates of relapse and treatment failure (Bayram et al., 2011). The use of new antibiotics, such as tigecycline, may hold future promise.

Treatment of human brucellosis needs antibiotics that can penetrate macrophages and can act in the acidic intracellular environment. *Brucella* is considered to be susceptible to the antibiotics recommended by the WHO for treatment of brucellosis. Relapses, at a rate of about 10%, usually occur in the first year after the infection, but they are caused in most cases by inadequate treatment (Pappas et al., 2005). Strains resistant to the main antimicrobial agents may emerge and lead to treatment inhibition (Marianelli et al., 2004).

Antimicrobial susceptibility testing for *Brucella* spp. is not generally recommended for routine microbiology laboratories except in life threatening organ involvement, and in the case of treatment failure and relapse (Bayram et al., 2011; King, 2001). Another problem with *Brucella*

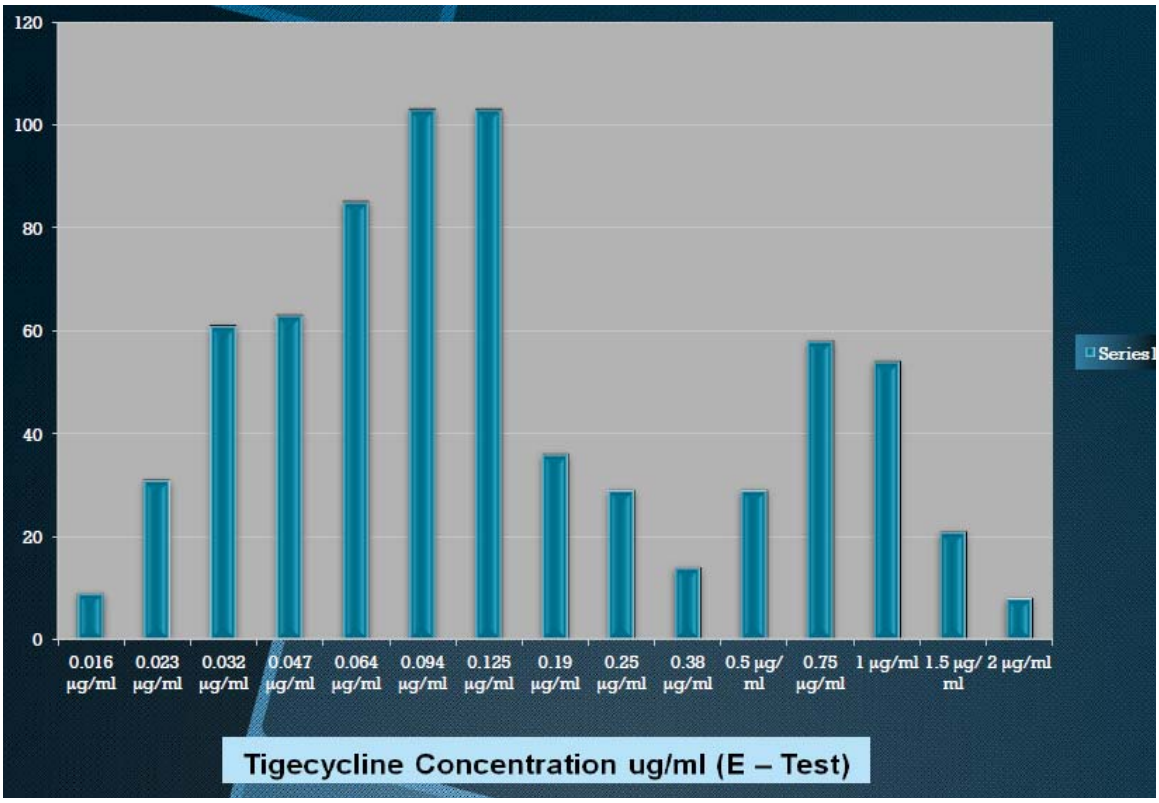

Figure 1. Brucella isolates vs. MIC values.

AST is the lack of standardization. Methods for MIC determination are described for potential bioterrorism agents including *Brucella* species by the CLSI. The CLSI proposes the microbroth dilution method using *Brucella* broth for *Brucella* spp. (Bayram et al., 2011). *In vitro* efficacy of antibiotics against *Brucella* spp. has usually been based on the determination of MIC values by micro broth dilution, agar dilution and E-test methods (Bayram et al., 2011; Shapiro et al., 2001). E-test method was found to be reliable, reproducible, less labor-intensive, less time-consuming and more practical than the broth micro dilution method (Köse et al., 2005; Bayram et al., 2011; Turkmani et al., 2006; Marianelli et al., 2007; CLSI, 2006; Gür, 1999). Therefore, E-test method was used in this study. E-test could be performed on Mueller-Hinton agar plates.

The *in vitro* activity of TIG, a new glycylcycline compound, against *Brucella* strains may hold future promise particularly in patients with multiple relapses on standard therapy. We found that TIG was effective against *Brucella*, reports suggests that TIG is more effective than rifampin (RIF), TMP-SMZ and streptomycin (STR) but was not as effective as doxycycline (DOX). Dizbay et al. (2011) reported TIG was more effective than RIF, SXT, STR, and DOX. Also, Kilic et al. (2007) found TIG had the least MIC_{50} and MIC_{90} values as compared to tetracycline (TET), and fluoroquinolones against *Brucella* strains isolated in Central Anatolia (Kilic et al., 2008).

Although, TIG has similar properties with TET, it has been reported that it is more potent than TET (Bayram et al., 2011; Zhanel et al., 2006; Livermore et al., 2005). TET is the mainstay of anti-brucellosis regimen. Therefore, Bayram et al. (2011) and Pappas et al. (2008) suggested that replacing DOX with more potent TIG might increase efficacy and reduce treatment duration.

In conclusion, antimicrobial resistance in *Brucella* isolates is still limited in Saudi Arabia. The results of this *in vitro* study suggest that TIG can be used as a therapeutic option in the treatment of brucellosis. Clinical trials are warranted to assess the real therapeutic potential of TIG in human brucellosis, particularly in countries with higher prevalence of treatment relapses and when combination therapy is needed (Bayram et al., 2011; Pappas et al., 2008; Mile et al., 2012).

Conflict of interest

The authors declare that they have no conflict of interest.

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