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ORIGINAL ARTICLE

Minimum inhibitory concentration values and problematic disk break points of tigecycline against vancomycin and/or high-level aminoglycoside-resistant enterococci



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KEYWORDS

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Abstract *Background:* Tigecycline is a new, semisynthetic glycolcycline. It is active against important multidrug resistant pathogens.

Aim: The purpose of this study was to investigate the sensitivity of multidrug-resistant enterococci to tigecycline, and to test the correlation between the minimal inhibitory concentration (MIC) and disk diffusion methods.

Materials and methods: The antimicrobial sensitivity of 108 multidrug-resistant isolates, which included 52 vancomycin-resistant enterococci (VRE) and 56 high-level aminoglycoside-resistant (HLAR) enterococci, was tested by the E test, broth microdilution test and disk diffusion methods.

Results: All of the isolates were sensitive to tigecycline, as determined by the E test and broth microdilution test. The MIC 90 value (0.19 µg/mL) of tigecycline for HLAR enterococci was higher than that for VRE (0.094 µg/mL). When results were evaluated according to species, the MIC values of tigecycline for *Enterococcus faecalis* were higher than those for the other species. Eleven (10.1%) isolates produced false resistance results (zone diameter ≤15 mm) by the disk diffusion method. These cases were classified as major errors. Eight (7.4%) isolates had intermediate sensitivity (sensitivity zone of 16 or 17 mm), which were classified as minor errors. The major and minor error percentages of HLAR enterococci (14.2% major, 10.7% minor error) were higher than those of VRE (5.7% major, 3.8% minor error). These results indicate that tigecycline is effective

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against multidrug-resistant enterococci. The sensitivity of multidrug-resistant enterococci to tigecycline should be investigated by MIC methods. The disk diffusion method causes major errors, especially for HLAR enterococci.

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1. Introduction

In recent years, the treatment of enterococcal infections has become more and more difficult because of the increasing antibiotic resistance of these organisms. Many enterococci are resistant to vancomycin, ampicillin, and high-level aminoglycosides, which are the most useful of the traditional antienterococcal antibiotics. Linezolid, daptomycin, and tigecycline are new antibiotics that are used to treat enterococcal infections. Tigecycline is a tetracycline derivative, and it has been reported to be effective against vancomycin-resistant enterococci (VRE) and tetracycline-resistant enterococci. However, the clinical data that are currently available are insufficient.^{1–5}

In this study, we investigated the sensitivity of multidrug-resistant enterococci to tigecycline. We also examined the correlation between the minimal inhibitory concentration (MIC) and disk diffusion methods.

2. Materials and methods

This study was performed using 108 strains of enterococci. The stool samples sent for various tests from patients with diarrhea or parasitic infection were inoculated on three brain–heart infusion agar plates containing 6 µg/mL vancomycin or 2000 µg/mL streptomycin or 500 µg/mL gentamicin.⁶ The growing colonies were examined by Gram staining and catalase test. Gram positive and catalase negative strains were tested for their ability to grow on 40% bile-esculin agar (Difco Laboratories, Detroit, USA) and in 6.5% NaCl broth (Becton, Dickinson and Company, Sparks, USA). The growing isolates on esculin agar containing 40% bile and in the 6.5% NaCl broth were identified at the species level using a RapID (Remel, Lenexa, KA, USA) test kit. All of the strains were again tested by agar screening method using brain–heart infusion agar plates containing 6 µg/mL vancomycin or 2000 µg/mL streptomycin or 500 µg/mL gentamicin for vancomycin and high level aminoglycoside resistance (streptomycin and gentamicin).⁶

Vancomycin resistant genes of VRE were determined by polymerase chain reaction (PCR). Bacterial DNA was extracted from all samples using a NucleoSpin DNA extraction kit (Macherey–Nagel, Duren, Germany) according to the manufacturer's instructions. PCR analysis was performed as previously described with minor modifications.⁷

Resistance to tetracycline and ampicillin was tested by the Bauer–Kirby disk diffusion method. Results were evaluated according to the CLSI criteria.⁶

The disk diffusion test for tigecycline was performed and evaluated according to the recommendations of European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (disk content: 15 µg tigecycline; inocula: McFarland standard 0.5; medium: Mueller–Hinton agar;

incubation: 18–20 h at 35 °C). The sensitivity zone diameters of enterococci for tigecycline were published as sensitive (≥ 18 mm) and resistant (≤ 15 mm) by EUCAST guidelines (2013).⁸

Tigecycline MIC break points were investigated by the E test and broth microdilution test.

E tests were performed according to the manufacturer's recommendations (AB Biodisk, Solna, Sweden). Inoculum suspensions with a turbidity equivalent to the 0.5 McFarland standard were prepared. The suspension was spread evenly onto a Muller Hinton agar plate. The E test tigecycline gradient strip was placed on the agar surface, and the plate was incubated at 35 °C for 18–20 h.

The broth microdilution test was performed according to CLSI guidelines. Mueller–Hinton II broth (BBL, Becton Dickenson, Sparks, MD) was freshly prepared and used within 24 h. Microplates (96 wells) containing serial dilutions of tigecycline (Wyeth Research, Pearl River, NY) (1 µg/ml, 0.5 µg/ml, 0.125 µg/ml, 0.0625 µg/ml, 0.0312 µg/ml, 0.0156 µg/ml) were prepared and each well was inoculated with test organisms to yield the appropriate density (10^5 CFU/ml) in 100 µl Mueller–Hinton broth (MHB) and plates were incubated for 24 h at 35 °C. The MIC was determined as well with the lowest drug concentration at which there was no visible growth.⁹

Enterococcus faecalis ATCC 29212 and *E. faecalis* ATCC 51299 were used for the quality control of susceptibility tests and PCR.

3. Results

Fifty-two of the isolates were VRE carrying the *vanA*, *vanC1*, or *vanC2–3* gene. Fifty-six isolates were high-level aminoglycoside-resistant (HLAR) enterococci. Forty-six of the HLAR enterococci isolates were resistant only to streptomycin, seven were resistant only to gentamicin, and three were resistant to both gentamicin and streptomycin. The species of the isolates were as follows: 11 *Enterococcus gallinarum*, 26 *Enterococcus casseliflavus*, 40 *E. faecalis*, 29 *Enterococcus faecium*, 1 *Enterococcus durans*, and 1 *Enterococcus avium*. VRE included 9 *E. casseliflavus*, 11 *E. faecalis*, 23 *E. faecium*, 8 *E. gallinarum*, and 1 *E. durans*. HLAR enterococci included 17 *E. casseliflavus*, 29 *E. faecalis*, 6 *E. faecium*, 3 *E. gallinarum*, and 1 *E. avium*.

The results of E test were consistent with broth microdilution tests. All of the isolates were sensitive to tigecycline according to MIC^{8–11} and the MIC values ranged from 0.023 to 0.125 µg/mL. The MIC 50 and MIC 90 values were 0.064 and 0.125 µg/mL, respectively. The MIC range for VRE was from 0.023 to 0.19 µg/mL. The MIC 50 and MIC 90 values were 0.064 and 0.094 µg/mL, respectively. The MIC range for HLAR enterococci was from 0.023 to 0.125 µg/mL, and the MIC 50 and MIC 90 values were 0.094 and 0.19 µg/mL,

respectively. Tigecycline MIC 50 and 90 values were higher for HLAR enterococci than for VRE. The MIC 50 and MIC 90 values of tigecycline are shown in Table 1. The MIC values for *E. faecalis* were higher than those for the other species. The MIC 50 and MIC 90 values for *E. faecalis* were 0.094 and 0.19 µg/mL, respectively.

All isolates had multiple resistances. Sixty-nine percent of them were sensitive to ampicillin. The sensitivity rates to tetracycline were low (20%). The antimicrobial sensitivities of the enterococci are listed in Table 2.

Although all of the isolates were sensitive to tigecycline according to MIC, the sensitivity zones of 19 (17.5%) isolates were less than 18 mm according to the disk diffusion method. The sensitivity zone diameters of enterococci for tigecycline were published as sensitive (≥18 mm) and resistant (≤15 mm) by EUCAST guidelines (2013).⁸ Eleven (10.1%) of these 19 isolates produced false resistance results (sensitivity zone diameters ≤15 mm) by the disk diffusion method. These cases were classified as major errors. The remaining eight (7.4%) isolates had intermediate sensitivity (sensitivity zone diameter of 16 or 17 mm), which were classified as minor errors. The major and minor error percentages of HLAR enterococci were higher than those of VRE (HLAR, 14.2% major errors and 10.7% minor errors; VRE, 5.7% major errors and 3.8% minor errors).

When evaluated on a species basis, *E. faecalis*, *E. casseliflavus*, *E. faecium*, and *E. gallinarum* had 15%, 7.6%, 6.8%, and 9% major errors, respectively. The major and minor errors of isolates are listed in Table 3.

4. Discussion

Tigecycline is active against a broad range of bacterial infections caused by important multidrug-resistant pathogens. It is a semisynthetic analog of tetracycline, and is effective against tetracycline-resistant enterococci. The two main mechanisms of bacterial resistance to tetracycline are active efflux of drugs (TetA-E, TetK) from inside the bacterial cell, and ribosomal protection (TetO, TetM). The N-alkyl glycyamido side chain at the carbon-9 position of tigecycline provides some biological advantages that differ from those of tetracycline. This side chain increases the lipid solubility of the drug and creates steric hindrance, which prevents tigecycline from cellular export by membrane-bound efflux proteins. Furthermore, the affinity to the binding site on the ribosome is increased. Tigecycline binds to ribosomes five times stronger than tetracycline. Consequently, tigecycline overcomes tetracycline resistance mechanisms.¹² In this study, 80% of isolates were resistant to tetracycline. Tigecycline was found to be effective against all of the tetracycline-resistant enterococci.

There are several reports about the activity of tigecycline against VRE. However, we were unable to find a published report about its activity against HLAR enterococci. We studied the effect of tigecycline on VRE and HLAR enterococci in this study. Thus, to the best of our knowledge, this is the first study to investigate the effects of tigecycline against HLAR enterococci.

In almost all previous studies, VRE isolates were sensitive to tigecycline or their resistance rates for tigecycline were very

Table 1 Minimum inhibitory concentration (MIC) values of tigecycline for enterococci.

Bacteria	MIC range (µg/ml)	MIC 50 (µg/ml)	MIC 90 (µg/ml)
High-level aminoglycoside-resistant <i>E. casseliflavus</i>	0.032–0.094	0.064	0.094
Vancomycin resistance <i>E. casseliflavus</i>	0.023–0.094	0.047	0.094
All of <i>E. casseliflavus</i>	0.023–0.125	0.064	0.094
High-level aminoglycoside-resistant <i>E. faecalis</i>	0.023–0.125	0.094	0.125
Vancomycin resistance <i>E. faecalis</i>	0.023–0.125	0.047	0.094
All of <i>E. faecalis</i>	0.023–0.125	0.094	0.19
High-level aminoglycoside-resistant <i>E. faecium</i>	0.032–0.125	0.047	0.064
Vancomycin resistance <i>E. faecium</i>	0.023–0.125	0.064	0.094
All of <i>E. faecium</i>	0.023–0.125	0.064	0.125
All of <i>E. gallinarum</i>	0.032–0.19	0.047	0.064
All of vancomycin-resistant enterococci	0.023–0.19	0.064	0.094
All of high-level aminoglycoside-resistant enterococci	0.023–0.125	0.094	0.19
All of 1 isolates	0.023–0.125	0.064	0.125

Table 2 Antibiotic susceptibility of enterococci.

Bacteria (n)	Antimicrobial drugs n (%)			
	Tetracycline n (%)	Vancomycin n (%)	Streptomycin n (%)	Gentamicin n (%)
<i>E. gallinarum</i> (11)	3(27)	3(27)	9(82)	8(73)
<i>E. casseliflavus</i> (26)	6(23)	12(46)	11(42)	21(81)
<i>E. faecalis</i> (40)	6(15)	27(68)	13(33)	34(85)
<i>E. faecium</i> (29)	6(21)	3(10)	24(83)	24(83)
<i>E. avium</i> (1)	0	1	0	1
<i>E. durans</i> (1)	1	0	1	1
All of 1 isolates	22(20)	46(43)	58(54)	89(82)

Table 3 Error rates of the disk diffusion method according to species.

Bacterial features (<i>n</i>)	Major error <i>n</i> (%)	Minor error <i>n</i> (%)	Total error <i>n</i> (%)
Vancomycin-resistant <i>E. casseliflavus</i> (9) ^a	1	0	1
Vancomycin-resistant <i>E. faecalis</i> (11)	0	1(0.9)	1(0.9)
Vancomycin-resistant <i>E. faecium</i> (23)	1(4.3)	1(4.3)	2(8.6)
Vancomycin-resistant <i>E. gallinarum</i> (8) ^a	1(12.5)	0	1(12.5)
Vancomycin-resistant <i>E. durans</i> (1)	0	0	0
Vancomycin-resistant Enterococci (52)	3(5.7)	2(3.8)	5(9.6)
High-level aminoglycoside-resistant <i>E. casseliflavus</i> (17)	1(5.8)	2(11.7)	3(17.6)
High-level aminoglycoside-resistant <i>E. faecalis</i> (29)	6(20.6)	4(13.7)	10(34.4)
High-level aminoglycoside-resistant <i>E. faecium</i> (6) ^a	1	0	1
High-level aminoglycoside-resistant <i>E. gallinarum</i> (3) ^a	0	0	0
High-level aminoglycoside-resistant <i>E. avium</i> (1)	0	0	0
High-level aminoglycoside-resistant enterococci (56)	8(14.2)	6(10.7)	14(25)
All of 1 isolates (108)	11(10.1)	8(7.4)	19(17.5)

^a Percentage was not entered because of the smallness of the number.

low. The MIC 90 values were reported to range from 0.0125 to 0.25 µg/mL.^{2,4,5,13,14} The results of this study confirmed the previous results related to VRE. All of our VRE isolates were sensitive to tigecycline, and the MIC 90 value was 0.094 µg/mL.

We observed that the MIC 90 value for HLAR enterococci (0.19 µg/mL) was higher than that for VRE (0.094 µg/mL). In addition, the MIC 90 value was higher for HLAR *E. faecalis* than for VR *E. faecalis*. The opposite was true for *E. faecium* (see Table 1). Unfortunately, the low number of HLAR *E. faecium* isolates (only 6 isolates) precluded any interpretation.

The discordance between the disk method and MIC break points has been reported in some previous studies. Hope et al. reported that 5% (major error) of sensitive enterococci by the agar dilution method were resistant by the disk diffusion method.¹⁵ We found a very high major error rate (10.1%) between the MIC and disk diffusion methods.

In the study by Liu et al., the major error, minor error, and total error for tigecycline in VRE were reported as 2.6%, 1.1%, and 4%, respectively.¹⁶ We observed major and minor error rates of 5.7% and 3.8%, respectively, for our VRE isolates. The major and minor error percentages of the HLAR enterococci (14.2% and 10.7%, respectively) were much higher than those of VRE. The major error rate peaked for HLAR *E. faecalis* (20.6%). Acceptable inter-method error rates are 3% for major error and 10% for minor error.¹⁷ Major error percentages were higher than acceptable in both our study and the study by Hope et al.¹⁵

These results indicate that tigecycline is effective against multidrug-resistant enterococci. The sensitivity of multidrug-resistant enterococci to tigecycline should be investigated by MIC methods. The disk diffusion method results in major errors, especially for multidrug-resistant HLAR enterococci.

Conflict of interest

No conflict of interest was declared by the authors.

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