

In Vitro Study to Determine the Activity of Tigecycline as Compared to Nine Comparator Antimicrobials Against Vancomycin Resistant and Sensitive *Enterococcus faecium* Isolates from the Tigecycline Evaluation Surveillance Trial (T.E.S.T.)

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REVISED ABSTRACT

Background: Vancomycin resistant enterococci pose serious clinical therapeutic challenges to clinicians with few effective agents at their disposal. Tigecycline (GAR-936), a member of a new class of antimicrobials (glycylcyclines), has shown to be very active against *Enterococcus faecium*, especially vancomycin resistant species with higher activity than currently available antimicrobials. The T.E.S.T. determined the in vitro activity of tigecycline compared with amoxicillin-clav, imipenem, ceftriaxone, levofloxacin, penicillin, vancomycin and linezolid against vancomycin resistant *Enterococcus faecium* (VRE) and vancomycin sensitive *Enterococcus faecium* (VSE) in hospitals worldwide. **Methods:** A total of 195 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Isolates were collected between January 2004 - November 2004. MIC's were determined by the local laboratory using broth microdilution panels from Dade Microscan according to NCCLS guidelines and manufacturer's instructions. **Results:** The in vitro activity of tigecycline was unaffected by resistance mechanisms responsible for the vancomycin resistant phenotype for *E. faecium*. Tigecycline MIC₅₀ and MIC₉₀ of 0.25 mcg/mL for VRE were 4 to 16 fold lower than minocycline (MIC₅₀ 1 mcg/mL; MIC₉₀ 8 mcg/mL), 256 fold lower than those of amoxicillin/clav, ceftriaxone, levofloxacin, (MIC₅₀ >64 mcg/mL; MIC₉₀ >64 mcg/mL) and 8 fold lower than linezolid (MIC₅₀ 2 mcg/mL; MIC₉₀ 2 mcg/mL). **Conclusion:** Tigecycline demonstrates potent in vitro activity against all strains of *E. faecium* and appears to be a promising antimicrobial agent against the vancomycin-resistant phenotype.

INTRODUCTION

Tigecycline is a novel antimicrobial with an expanded broad-spectrum of activity from a new class of compounds, glycylcyclines. Tigecycline inhibits protein synthesis by binding to the 30S

ribosomal subunit and although it is perceived to be bacteriostatic, its anti-bacterial activity is significant and has shown some bactericidal activity against key targeted pathogens [1,2].

Tigecycline was developed to provide activity against tetracycline- and multi-drug-resistant gram-positive pathogens and has demonstrated significant broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative microorganisms [2-3].

Tigecycline has shown potent activity against animal models infected with selected strains of multi-drug resistant *Enterococcus faecium* [4, 5] and a limited number of clinical isolates of *E. faecium* with various vanA, -B and -C genes [6]. Since current treatment options against vancomycin resistant *E. faecium* are largely limited to doxycycline, quinupristin/dalfopristin and linezolid, the activity of tigecycline was prospectively compared to these antimicrobial agents in 195 clinical strains of *E. faecium*.

MATERIALS & METHODS

All isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, fluids and few other defined sources. Only one isolate per patient was accepted.

Clinical isolates were collected tested between January 2004 - November 2004 from 20 study centers in 6 countries.

Antimicrobial agents tested with concentrations (expressed in mcg/ml) were: amoxicillin/clavulanic acid (0.03-8); piperacillin/tazobactam (0.25-16); levofloxacin (0.06-32); ceftriaxone (0.03-64); linezolid (0.5-8); minocycline (0.25-8); vancomycin (0.12-32); ampicillin (0.06-16); penicillin (0.06-8); tigecycline (0.008-16); imipenem (0.12-16). MIC

interpretive criteria followed published guidelines established by the NCCLS where applicable [7]. Tigecycline tentative breakpoints (in units of mcg/mL) are defined as susceptible ≤ 2; intermediate = 4; and resistant ≥ 8.

Isolates were identified to genus and species at each site by the local laboratory. Isolates were tested by the local laboratory.

Organism collection, transport, confirmation of organism identification, as well as, construction and management of a centralized database was coordinated by Laboratories International for Microbiology Studies (LIMS).

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RESULTS

Results are shown in the following tables.

Table 1. In Vitro Activity of Tigecycline and Comparative Agents Against 195 Strains of *Enterococcus faecium*.

| Organism Name | Drug | %SUS | %INT | %RES | MICs (mcg/mL) | |
|-------------------------------------|--------------|-------|------|------|---------------|-------|
| | | | | | MIC50 | MIC90 |
| <i>Enterococcus faecium</i> (n=195) | Tigecycline | 100.0 | 0.0 | 0.0 | 0.06 | 0.12 |
| | Ampicillin | 9.5 | 0.0 | 90.5 | >16 | >16 |
| | Levofloxacin | 9.5 | 0.0 | 90.5 | >32 | >32 |
| | Linezolid | 96.4 | 1.2 | 2.4 | 2 | 2 |
| | Minocycline | 60.7 | 28.6 | 7.1 | 0.5 | 8 |
| | Penicillin | 8.3 | 0.0 | 91.7 | >8 | 16 |
| Vancomycin | 40.5 | 1.2 | 58.3 | >32 | >32 | |

* Breakpoints of all antimicrobial agents are defined by the NCCLS 2004, document M100-S14; Tigecycline tentative breakpoints (in mcg/mL) defined as susceptible ≤ 2; intermediate = 4; resistant ≥ 8.

Table 2. In Vitro Activity of Tigecycline and Comparative Agents Against 195 strains of Vancomycin-Sensitive and -Resistant *Enterococcus faecium*

| Organism Name | Drug | %SUS | %INT | %RES | MICs (mcg/mL) | |
|--|--------------|-------|-------|-------|---------------|-------|
| | | | | | MIC50 | MIC90 |
| Vancomycin-Sensitive <i>Enterococcus faecium</i> (n=81) | Tigecycline | 100.0 | 0.0 | 0.0 | 0.06 | 0.12 |
| | Ampicillin | 22.9 | 0.0 | 77.1 | >16 | >16 |
| | Levofloxacin | 22.9 | 0.0 | 77.1 | >32 | >32 |
| | Linezolid | 94.3 | 2.9 | 2.9 | 2 | 2 |
| | Minocycline | 68.6 | 14.3 | 14.3 | 0.5 | >8 |
| | Penicillin | 20.0 | 0.0 | 80.0 | >8 | 16 |
| Vancomycin-Resistant <i>Enterococcus faecium</i> (n=114) | Tigecycline | 100.0 | 0.0 | 0.0 | 0.06 | 0.06 |
| | Ampicillin | 0.0 | 0.0 | 100.0 | >16 | >16 |
| | Levofloxacin | 0.0 | 0.0 | 100.0 | >32 | >32 |
| | Linezolid | 98.0 | 0.0 | 2.0 | 2 | 2 |
| | Minocycline | 55.1 | 38.8 | 2.0 | 4 | 8 |
| | Penicillin | 0.0 | 0.0 | 100.0 | >8 | 16 |
| Vancomycin | 0.0 | 0.0 | 100.0 | >32 | >32 | |

* Breakpoints of all antimicrobial agents are defined by the NCCLS 2004, document M100-S14; Tigecycline tentative breakpoints (in mcg/mL) defined as susceptible ≤ 2; intermediate = 4; resistant ≥ 8.

Table 3. Frequency Distribution (n) and Cumulative Percent Inhibition (%) at each MIC (mcg/mL) for Tigecycline and Comparative Agents Against 81 Vancomycin-Sensitive *Enterococcus faecium*

| Drug | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | >64 |
|--------------|------|------|------|------|-------|------|------|------|-------|------|------|-------|-------|
| Tigecycline | 23 | 38 | 14 | 5 | 1 | | | | | | | | |
| Ampicillin | 28.6 | 74.3 | 91.4 | 97.1 | 100.0 | 9 | 7 | 2 | | | | 63 | |
| Levofloxacin | | | | | | 11.4 | 20.0 | 22.9 | | | | 100.0 | |
| Linezolid | | | | | | 1 | 5 | 13 | 1 | 1 | 8 | 52 | |
| Minocycline | | | | | | 2.9 | 8.6 | 22.9 | 25.7 | 28.6 | 37.1 | 100.0 | |
| Penicillin | | | | | | 15 | 64 | 1 | 1 | | | | |
| Vancomycin | | | | | | 17.1 | 94.3 | 97.1 | 100.0 | | | | |
| | | | | | | 22 | 24 | 1 | 7 | 13 | | | 13 |
| | | | | | | 28.6 | 57.1 | 60.0 | 62.9 | 71.4 | 85.7 | 100.0 | |
| | | | | | | | | | | | | | 63 |
| | | | | | | | | | | | | | 97.1 |
| | | | | | | | | | | | | | 100.0 |
| | | | | | | | | | | | | | 1 |
| | | | | | | | | | | | | | 100.0 |
| | | | | | | | | | | | | | 1 |
| | | | | | | | | | | | | | 100.0 |

Dividing lines represent MIC₉₀ demarcation.

Table 4. Frequency Distribution (n) and Cumulative Percent Inhibition (%) at each MIC (mcg/mL) for Tigecycline and Comparative Agents Against 114 Vancomycin-Resistant *Enterococcus faecium*

| Drug | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | >64 |
|--------------|------|------|-------|------|-----|---|---|---|---|----|----|-------|
| Tigecycline | 53 | 62 | 9 | | | | | | | | | |
| Ampicillin | 46.9 | 91.8 | 100.0 | | | | | | | | | |
| Levofloxacin | | | | | | | | | | | | 114 |
| Linezolid | | | | | | | | | | | | 100.0 |
| Minocycline | | | | | | | | | | | | 5 |
| Penicillin | | | | | | | | | | | | 12 |
| Vancomycin | | | | | | | | | | | | 14.3 |
| | | | | | | | | | | | | 100.0 |
| | | | | | | | | | | | | 2 |
| | | | | | | | | | | | | 84 |
| | | | | | | | | | | | | 98.0 |
| | | | | | | | | | | | | 100.0 |
| | | | | | | | | | | | | 3 |
| | | | | | | | | | | | | 45 |
| | | | | | | | | | | | | 3 |
| | | | | | | | | | | | | 109 |
| | | | | | | | | | | | | 95.9 |
| | | | | | | | | | | | | 100.0 |
| | | | | | | | | | | | | 3 |
| | | | | | | | | | | | | 111 |
| | | | | | | | | | | | | 2.0 |
| | | | | | | | | | | | | 100.0 |

Dividing lines represent MIC₉₀ demarcation.

Figure 1. In Vitro Activity of Tigecycline and Comparators Against 81 Vancomycin-Sensitive *Enterococcus faecium* Showing Cumulative Percent Inhibited (%) at Each MIC (mcg/ml)

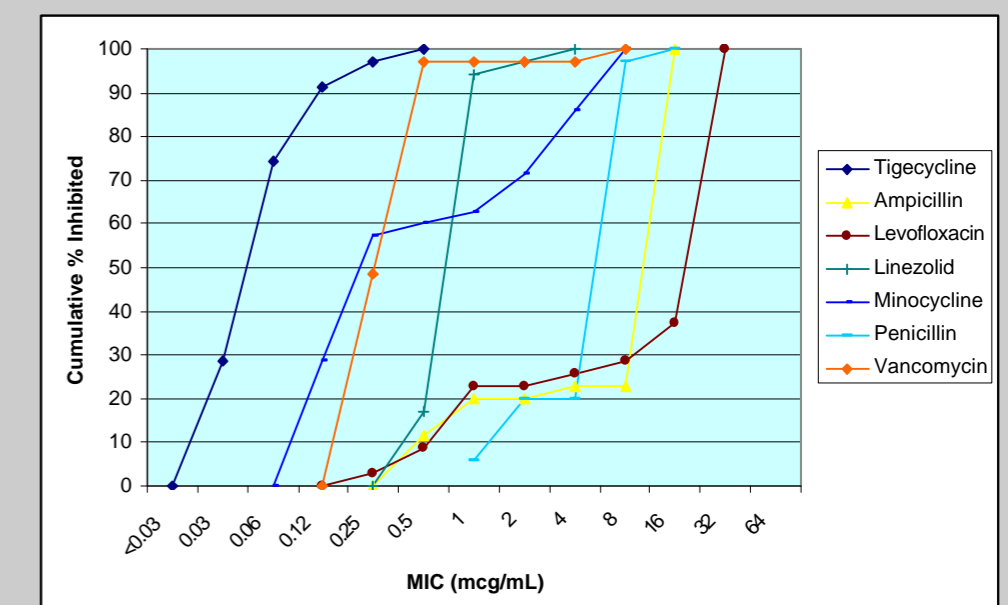
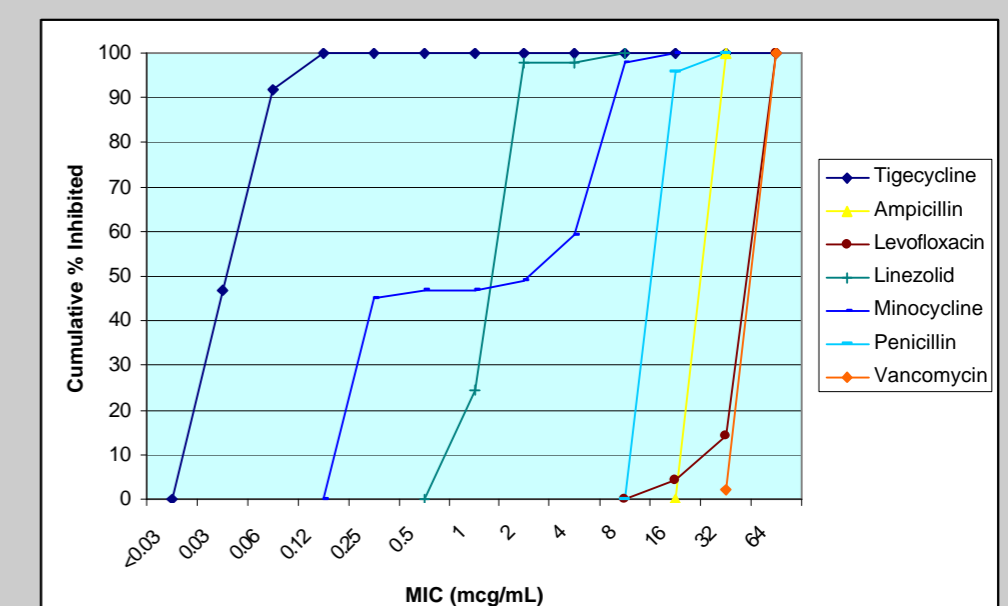


Figure 2. In Vitro Activity of Tigecycline and Comparators Against 114 Vancomycin-Resistant *Enterococcus faecium* Showing Cumulative Percent Inhibited (%) at Each MIC (mcg/ml)



CONCLUSIONS

- Tigecycline had the lowest MIC90 of all comparative agents against both vancomycin-sensitive (0.12 mcg/mL) and vancomycin-resistant (0.06 mcg/mL) *Enterococcus faecium*.
- Tigecycline MIC₉₀s were 32, 128 and 256 fold more potent against vancomycin-resistant *Enterococcus faecium* than linezolid, minocycline and levofloxacin, respectively.
- Tigecycline exhibits potent in vitro activity against *Enterococcus faecium* without regard to vancomycin phenotype.