Antibiotic Activity against Vancomycin Resistant Enterococci from the Tigecycline Evaluation Surveillance Trials (TEST) Program 2006

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REVISED ABSTRACT

Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the in vitro activity of tigecycline compared to amoxicillin-clavulanic acid, piperacillin-tazobactam, levofloxacin, linezolid (LZD), minocycline, vancomycin (VAN), ampicillin (AM), penicillin, and imipenem against VRE collected in 272 medical centers globally between 2004-2006. **Methods:** 629 VRE clinical isolates (92 *E. faecalis*, 537 *E. faecium*) were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines. Tigecycline susceptible breakpoint was defined as ≤0.25 mcg/mL for VRE. **Results:** %Susceptible of all VRE to TIG, LZD, and MIN were 100, 97.0, and 72.0, respectively. For *E. faecalis* strains, the most active drugs were TIG (100%), AM (100%), P (100%), and LZD (98.2%). For *E. faecium*, the three most active drugs were TIG (100%), LZD (96.3%), and MIN (73.6%). There were significant differences in VRE rates between North America (22.4%), Europe (4.0%), Asia/Pacific (9.6%), and Latin America (10.1%); and the Middle East reported 0/17 *E. faecalis* and 1/11 *E. faecium* as resistant. **Conclusion:** TIG exhibited outstanding in vitro activity against VRE, inhibiting 100% of strains with MICs ≤0.25 mcg/mL, surpassing LZD as the most active drug in this study. The exceptionally broad spectrum of TIG, which includes many other multi-resistant gram-positive and gram-negative bacteria in addition to VRE, should make it a very attractive addition to hospital formularies.

INTRODUCTION

Tigecycline is a novel antimicrobial with expanded broad-spectrum activity from a new class of compounds, the glycylcyclines. Tigecycline inhibits protein synthesis by binding to the 30S ribosomal subunit. 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 [2-4].

Tigecycline resistance is very infrequent and is also difficult to induce in the laboratory [5, 6] with a selection frequency observed at less than 10^{-9} [3, 5, 7]. With the exception of *P. aeruginosa*, tetracycline-resistant bacteria with either tetracycline efflux pumps or ribosomal protective features are sensitive to tigecycline [2-4, 7-11]. Tigecycline has shown to be a highly effective against multi-resistant *Acinetobacter* spp., particularly *A. baumannii* that are commonly associated with serious nosocomial infections. Similar activity has been observed against *Enterobacteriaceae*, even extended-spectrum beta-lactamase (ESBL) and AmpC producing strains [10]. Tigecycline has demonstrated MIC_{90} values of ≤0.5 mcg/ml against methicillin-resistant *Staphylococcus aureus* (MRSA) and other gram-positive organisms [2, 4-6]. Tigecycline has shown potent activity against animal models infected with selected strains of multi-drug resistant *Enterococcus faecium* and *Enterococcus faecalis* [4, 5] with diverse genotypes van-A, -B and -C [6].

This study was designed to better define the in vitro activity of tigecycline in vancomycin-resistant enterococci clinical isolates collected from 272 study centers worldwide.

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. Isolates were identified to genus and species by the local laboratory. Each site tested the isolates using broth microdilution.
- Clinical isolates (n=3,925) of *Enterococcus* spp were collected tested between January 2004 - December 2006 from 272 study centers in 34 countries.
- Custom broth microdilution panels were supplied by MicroScan (Dade MicroScan, Sacramento, CA, USA) with the following antimicrobial agents and concentrations (expressed in mcg/ml): amoxicillin/clavulanic acid (0.12-32); piperacillin/tazobactam (0.06-128); levofloxacin (0.008-8); ceftriaxone (0.06-64); cefepime (0.5-32); ampicillin (0.5-32); amikacin (0.5-64); minocycline (0.5-16); ceftazidime (8-32); tigecycline (0.008-16); and imipenem (0.06-16).
- MIC interpretive criteria for all drugs except tigecycline followed published guidelines established by the CLSI where applicable [12]. MIC interpretive criteria for tigecycline followed criteria established by the Federal Drug Administration (FDA, United States, 2005) where applicable [13].
- Quality control of broth microdilution panels followed manufacture's and NCCLS guidelines using the following ATCC strains: *Enterococcus faecalis* ATTC 29212; *Escherichia coli* ATCC 25922; *Klebsiella pneumoniae* ATCC 700603 (positive ESBL control); *Haemophilus influenzae* ATCC 49247; *Haemophilus influenzae* ATCC 49766; *Staphylococcus aureus* ATCC 29213; *Streptococcus pneumoniae* ATCC 49619; and *Pseudomonas aeruginosa* ATCC 27853.
- The collection and transportation of organisms and the confirmation of identification, as well as, construction and management of a centralized database were conducted and coordinated by Laboratories International for Microbiology Studies (LIMS), a subsidiary of International Health Management Associates, Inc. (IHMA, Schaumburg, IL, USA).

REFERENCES

1 Sum, P.E. and P. Petersen, *Synthesis and structure-activity relationship of novel glycylcycline derivatives leading to the discovery of GAR-936*. Bioorg Med Chem Lett, 1999. 9(10): p. 1459-62.
2 Abbanat, D., M. Macielag, and K. Bush, *Novel antibacterial agents for the treatment of serious Gram-positive infections*. Expert Opin Investig Drugs, 2003. 12(3): p. 379-99.
3 Betriu, C., et al., *In vitro activities of tigecycline (GAR-936) against recently isolated clinical bacteria in Spain*. Antimicrob Agents Chemother, 2002. 46(3): p. 892-5.
4 Gales, A.C. and R.N. Jones, *Antimicrobial activity and spectrum of the new glycylcycline, GAR-936 tested against 1,203 recent clinical bacterial isolates*. Diagn Microbiol Infect Dis, 2000. 36(1): p. 19-36.
5 Henwood, C.J., et al., *Antibiotic resistance among clinical isolates of Acinetobacter in the UK, and in vitro evaluation of tigecycline (GAR-936)*. J Antimicrob Chemother, 2002. 49(3): p. 479-87.
6 Chopra, I., *New developments in tetracycline antibiotics: glycylcyclines and tetracycline efflux pump inhibitors*. Drug Resist Updat, 2002. 5(3-4): p. 119-25.
7 Projan, S.J., *Preclinical pharmacology of GAR-936, a novel glycylcycline antibacterial agent*. Pharmacotherapy, 2000. 20(9 Pt 2): p. 219S-223S; discussion 224S-228S.
8 Biedenbach, D.J., M.L. Beach, and R.N. Jones, *In vitro antimicrobial activity of GAR-936 tested against antibiotic-resistant gram-positive blood stream infection isolates and strains producing extended-spectrum beta-lactamases*. Diagn Microbiol Infect Dis, 2001. 40(4): p. 173-7.
9 Patel, R., et al., *In vitro activity of GAR-936 against vancomycin-resistant enterococci, methicillin-resistant Staphylococcus aureus and penicillin-resistant Streptococcus pneumoniae*. Diagn Microbiol Infect Dis, 2000. 38(3): p. 177-9.
10 Petersen, P.J., et al., *In vitro and in vivo antibacterial activities of a novel glycylcycline, the 9-t-butylglycylamido derivative of minocycline (GAR-936)*. Antimicrob Agents Chemother, 1999. 43(4): p. 738-44.
11 Petersen, P.J., et al., *In vitro and in vivo activities of tigecycline (GAR-936), daptomycin, and comparative antimicrobial agents against glycopeptide-intermediate Staphylococcus aureus and other resistant gram-positive pathogens*. Antimicrob Agents Chemother, 2002. 46(8): p. 2595-601.
12 *Clinical Laboratory Standards Institute (CLSI)*. Performance Standards for Antimicrobial Susceptibility Testing; 16th Informational Supplement. CLSI document M100-S16. Wayne, PA, 2006.
13 Tygacil®, 2005. Tigecycline FDA package insert.

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RESULTS

The results are listed in the following tables.

Table 1. In vitro activity of tigecycline and comparative agents against *E. faecalis* and *E. faecium* combined and vancomycin-resistant *Enterococci* (VRE) by Region.*

Region VRE/Total N (%)	Drug	*E. faecalis*, *E. faecium* Combined %Sus	*E. faecalis*, *E. faecium* Combined MIC_{90}	Vancomycin-Resistant *Enterococci*‡ %Sus	Vancomycin-Resistant *Enterococci*‡ MIC_{90}
Global	Tigecycline	100	0.06	100	0.12
629/3831(16.4)	Ampicillin	77.1	1	17	>16
	Levofloxacin	48.2	8	1.3	>32
	Linezolid	97.7	2	97	2
	Minocycline	52.4	4	72	8
	Penicillin	76.6	2	16.1	>8
	Vancomycin	83.1	1	0	>32
Africa	Tigecycline	100	0.06	--	--
0/64(0)	Ampicillin	93.8	1	--	--
	Levofloxacin	64.1	1	--	--
	Linezolid	100	2	--	--
	Minocycline	29.7	8	--	--
	Penicillin	95.3	2	--	--
	Vancomycin	100	1	--	--
Asia / Pacific Rim	Tigecycline	100	0.06	100	0.12
18/188(9.6)	Ampicillin	75.5	1	0	>16
	Levofloxacin	50	4	0	>32
	Linezolid	97.9	2	100	2
	Minocycline	43.1	8	83.3	8
	Penicillin	74.5	2	5.6	>8
	Vancomycin	90.4	1	0	>32
Europe	Tigecycline	100	0.12	100	0.12
36/899(4.0)	Ampicillin	81.5	1	36.1	>16
	Levofloxacin	58.8	1	8.3	>32
	Linezolid	96	2	94.4	2
	Minocycline	48.1	8	83.3	8
	Penicillin	80.4	2	30.6	>8
	Vancomycin	95.8	1	0	>32
Latin America	Tigecycline	100	0.12	100	0.12
16/158(10.1)	Ampicillin	89.2	1	12.5	>16
	Levofloxacin	68.4	1	0	>32
	Linezolid	94.3	2	100	2
	Minocycline	52.5	4	81.3	>8
	Penicillin	89.9	2	18.8	>8
	Vancomycin	89.9	1	0	>32
Middle East	Tigecycline	100	0.06	100	0.12
1/28(3.6)	Ampicillin	64.3	2	0	>16
	Levofloxacin	39.3	32	0	>32
	Linezolid	96.4	2	100	2
	Minocycline	39.3	8	100	≤0.25
	Penicillin	60.7	8	0	>8
	Vancomycin	96.4	1	0	>32
North America	Tigecycline	100	0.06	100	0.12
558/2494(22.4)	Ampicillin	74.5	1	16.5	>16
	Levofloxacin	42.7	16	0.9	>32
	Linezolid	98.4	2	97	2
	Minocycline	55.5	4	70.6	8
	Penicillin	74.2	2	15.4	>8
	Vancomycin	76.9	1	0	>32

* Susceptibilities are defined in CLSI document M100-S16 (2006) where applicable. Tigecycline breakpoints are defined in FDA package insert (Tygacil®, 2005) as susceptible less than or equal to 0.25 mcg/mL for vancomycin-susceptible *E. faecalis*. This breakpoint was expanded to include all enterococci for comparative purposes only.
‡ Analysis of VRE was performed only on the two major species, *E. faecalis* and *E. faecium*.

Table 2. In vitro activity (mcg/mL and % susceptible) of tigecycline and comparative agents against 3,925 enterococci clinical isolates*.

Organism	Drug	%Sus	%Int	%Res	MIC (mcg/mL) MIC_{50}	MIC (mcg/mL) MIC_{90}
Enterococcus spp† (n=3,925)	Tigecycline	100	0	0	0.06	0.12
	Ampicillin	77	0	23	1	>16
	Levofloxacin	48.6	1.3	50	8	>32
	Linezolid	97.6	2.4	0	2	2
	Minocycline	52.8	36.5	10.7	4	>8
	Penicillin	76.5	0	23.5	2	>8
	Vancomycin	83	0.6	16.5	1	>32
Vancomycin-Resistant *enterococci*‡ (VRE, n=629)	Tigecycline	100	0	0	0.06	0.12
	Ampicillin	17	0	83	>16	>16
	Levofloxacin	1.3	0.2	98.6	>32	>32
	Linezolid	97	3	0	2	2
	Minocycline	72	21	7	≤0.25	8
	Penicillin	16.1	0	83.9	>8	>8
	Vancomycin	0	0	100	>32	>32
E. faecalis (n=2,779)	Tigecycline	100	0	0	0.12	0.12
	Ampicillin	100	0	0	1	1
	Levofloxacin	61.5	0.9	37.6	1	>32
	Linezolid	98.2	1.8	0	2	2
	Minocycline	44.4	43.9	11.7	8	>8
	Penicillin	100	0	0	2	4
	Vancomycin	96.4	0.3	3.3	1	2
E. faecium (n=1,052)	Tigecycline	100	0	0	0.06	0.12
	Ampicillin	16.5	0	83.5	>16	>16
	Levofloxacin	13.2	2.2	84.6	>32	>32
	Linezolid	96.3	3.7	0	2	2
	Minocycline	73.6	18	8.5	≤0.25	8
	Penicillin	14.7	0	85.3	>8	>8
	Vancomycin	47.9	1	51	32	>32

[a] Susceptibilities are defined in CLSI document M100-S16 (2006) where applicable. Tigecycline breakpoints are defined in FDA package insert (Tygacil®, 2005) as susceptible less than or equal to 0.25 mcg/mL for vancomycin-susceptible *E. faecalis*. This breakpoint was expanded to include all enterococci for comparative purposes only.
† This combined group includes *E. faecalis* (2,779); *E. faecium* (1,052); *E. durans* (20); *E. avium* (26); *E. casseliflavus* (27); non-speciated enterococci (7); *E. raffinosus* (6); E. Group D (4); *E. gallinarum* (2); and *E. hirae* (2).
‡ Analysis of VRE was performed only on the two major species, *E. faecalis* and *E. faecium*.

CONCLUSIONS

- Global VRE percentage rate was demonstrated to be 16.4% (for *E. faecalis* and *E. faecium*) in this surveillance of 272 sites from 34 countries. The percentage of VRE varied regionally from a low of 0% (0/64) in Africa's 2 reporting centers to high of 22.4% (558/2494) in 139 reporting sites from North America.
- Tigecycline demonstrated potent in vitro activity against all vancomycin-resistant enterococci with MIC_{90} values of 0.12 mcg/mL and inhibiting 100% of VRE isolates. Tigecycline's activity against VRE was superior to even that of linezolid, which had an MIC_{90} of 2 mcg/ml, and inhibited 97% of the isolates.
- The in vitro activity of tigecycline in this study suggests that tigecycline is effective against vancomycin-resistant *E. faecalis* or *E. faecium* that may be resistant to linezolid.