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In Vitro Evaluation of Tigecycline Against 288 Recent Isolates of Vancomycin-Resistant Enterococci (VRE) - T.E.S.T. 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, ceftriaxone, linezolid (LZD), minocycline, vancomycin (VAN), ampicillin (AM), penicillin (P), and imipenem against VRE collected from hospitals globally throughout 2004-2006.
Methods: 288 VRE (38 *Enterococcus faecalis*, 250 *Enterococcus faecium*) clinical isolates 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 with tigecycline susceptible breakpoint defined as ≤0.25 mcg/mL.
Results: %Susceptible of all VRE to TIG, LZD, and MIN were 100, 96.5, and 66.7, respectively. For *E. faecalis* strains, the three most active drugs were TIG (100%), LZD (100%), P (100%) and AM (100%). For *E. faecium*, the three most active drugs were TIG (100%), LZD (96%), and MIN (68.4%). There were significant differences in VRE rates between North America (*E. faecalis* 3.5%, *E. faecium* 65.5%), Europe (*E. faecalis* 1.5%, *E. faecium* 11.5%), Middle East (*E. faecalis* 0%, *E. faecium* 9.1%), Latin America (*E. faecalis* 4.5%, *E. faecium* 33.3%) and Asia (*E. faecalis* 0%, *E. faecium* 9.1%)
Conclusion: TIG exhibited potent in vitro activity against VRE, inhibiting 100% of strains with MICs ≤0.25 mcg/mL (MIC_{90} = 0.12), 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, will make it an attractive addition to hospital formularies.

INTRODUCTION

Tigecycline (formerly GAR-936) is a member of a new class of antimicrobial agents, the glycylcyclines. This synthetic analogue of the tetracyclines exhibits significant antibacterial activity that is both bacteriostatic and, in certain instances, bactericidal with killing activity that is as much as fourfold better than vancomycin and daptomycin [1, 2]. The development of tigecycline is important in that it and other glycylcyclines are active against bacterial strains carrying either or both of the two major forms of tetracycline resistance: efflux and ribosomal protection. Certain substituents at the 9-position of the tetracycline molecule restored activity against bacteria harboring genes encoding either or both efflux and ribosomal protection. A single chemical modification of tigecycline overcomes the two molecularly distinct forms of resistance while maintaining activity against susceptible gram-positive, gram-negative, aerobic, and anaerobic bacteria [3]. Furthermore, resistance to tigecycline is difficult to produce even in the laboratory.

Previous studies have demonstrated excellent in vitro activity for tigecycline against clinical and laboratory strains of Gram-positive and -negative bacteria with MIC_{90}s at or below 2 mcg/mL, including difficult-to-treat methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE) and extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* [4-6, 7]. The relatively limited treatment options for infections due to VRE in particular have led to development of new compounds with activity against these problematic pathogens. This study was undertaken to document the in vitro activity of tigecycline against a significant number of clinical isolates of VRE from North America, Europe, Asia/Pacific, Latin America, and the Middle East. This study is part of the larger ongoing global Tigecycline Evaluation and Surveillance Trials (T.E.S.T.) program.

MATERIALS & METHODS

- All isolates were derived from blood, respiratory tract, urine, skin, wound, body fluids and other defined sources. Only one isolate per patient was accepted into the study. Clinical isolates were collected and tested between January 2004 - January 2005 from 150 sites in 27 countries in North America, Europe, Asia/Pacific, Latin America, and the Middle East. Isolates were identified to the species level and tested at each site by the participating laboratory.
- Organism collection, transport, confirmation of organism identification, and development and management of a centralized database were coordinated by Laboratories International for Microbiology Studies (LIMS), a division of International Health Management Associates, Inc. located in Schaumburg, IL, USA.
- All organisms were deemed clinically significant by local participant criteria. Isolate inclusion was independent of medical history, antimicrobial use, age or gender. All sites identified each study isolate utilizing local laboratory site criteria.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

- Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [8]. Tigecycline was supplied by Wyeth Pharmaceuticals (Collegeville, PA, USA). All other agents were supplied by the panel manufacturer, MicroScan (Dade Behring Inc., West Sacramento, CA, USA). The following antimicrobial agents and dilution ranges (expressed in mcg/mL) were included on the panels: amoxicillin/clavulanic acid (0.12/0.06-32/16); ampicillin (0.06-16); imipenem (0.06-16); linezolid (0.5-8); levofloxacin (0.008-8); minocycline (0.5-16); tigecycline (0.008-16); penicillin (0.06-8); piperacillin/tazobactam (0.06/4-128/4) and vancomycin (0.12-32). MIC interpretive criteria followed published guidelines established by the Clinical and Laboratory Standards Institute [9] and the recent US Food and Drug Administration package insert for tigecycline [10], where applicable.
- Quality controls (QC) were performed by each testing site on each day of testing using the corresponding ATCC control strains: *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI (2005) guidelines [9].

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RESULTS

Table 1. Regional variation in rates of vancomycin resistance among 1,891 *E. faecalis* and *E. faecium* isolates.

Region	Vancomycin-resistant *E. faecalis* n/total (%)	Vancomycin-resistant *E. faecium* n/total (%)
North America	31/890 (3.5%)	232/354 (65.5%)
Europe	6/392 (1.5%)	14/122 (11.5%)
Latin America	1/22 (4.5%)	1/3 (33.3%)
Asia / Pacific Rim	0/65 (0%)	2/22 (9.1%)
Middle East	0/9 (0%)	1/11 (9.1%)
Global	38/1379 (2.8%)	250/512 (48.8%)

Table 2. In vitro activity of tigecycline and comparative antimicrobial agents against 38 vancomycin-resistant *E. faecalis* isolates from a global population.

Organism (N)	Drug	MIC (mcg/mL) MIC_{50}	MIC_{90}	Range	%Sus [a]
E. faecalis (VRE) (n=38)	Tigecycline	0.06	0.12	0.03 - 0.25	100
	Ampicillin	1	2	0.25 - 4	100
	Levofloxacin	32	>32	1 - >32	10.5
	Linezolid	1	2	≤0.5 - 2	100
	Minocycline	4	8	≤0.25 - >8	55.3
	Penicillin	2	8	0.5 - 8	100
	Vancomycin	>32	>32	32 - >32	0

[a]Interpretive criteria as defined by CLSI, M100-S15 (2005), where applicable; Tigecycline FDA breakpoints for *enterococci* are approved for vancomycin-susceptible *E. faecalis*, only; susceptibilities for all other *enterococci* are entered for comparison purposes only. [10]

Table 3. In vitro activity of tigecycline and comparative antimicrobial agents against 250 vancomycin-resistant *E. faecium* isolates from a global population.

Organism (N)	Drug	MIC (mcg/mL) MIC_{50}	MIC_{90}	Range	%Sus [a]
E. faecium (VRE) (n=250)	Tigecycline	0.06	0.12	0.015 - 0.25	100
	Ampicillin	>16	>16	0.5 - >16	4.4
	Levofloxacin	>32	>32	2 - >32	1.2
	Linezolid	2	2	≤0.5 - 4	96
	Minocycline	0.5	8	≤0.25 - >8	68.4
	Penicillin	>8	>8	2 - >8	2
	Vancomycin	>32	>32	32 - >32	0

[a]Interpretive criteria as defined by CLSI, M100-S15 (2005), where applicable; Tigecycline FDA breakpoints for *enterococci* are approved for vancomycin-susceptible *E. faecalis*, only; susceptibilities for all other *enterococci* are entered for comparison purposes only. [10]

Table 4. Frequency distribution and cumulative % inhibition for tigecycline and comparators vs. 38 isolates of vancomycin-resistant *Enterococcus faecalis* from a global population.

MIC n/cum%	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Tigecycline	4 10.5	21 65.8	11 94.7	2 100.0									
Ampicillin				2 5.3	9 28.9	21 84.2	4 94.7	2 100.0					
Levofloxacin						3 7.9	1 10.5			7 28.9	13 63.2	14 100.0	
Linezolid					2 5.3	22 63.2	14 100.0						
Minocycline				11 28.9			3 36.8	7 55.3	14 92.1	3 100.0			
Penicillin					1 2.6	5 15.8	17 60.5	11 89.5	4 100.0				
Vancomycin											4 10.5	34 100.0	

Table 5. Frequency distribution and cumulative % inhibition for tigecycline and comparators vs. 250 isolates of vancomycin-resistant *Enterococcus faecium* from a global population.

MIC n/cum%	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Tigecycline	2 0.8	115 46.8	103 88.0	29 99.6	1 100									
Ampicillin						2 0.8	2 1.6		2 2.4	5 4.4	4 6.0	235 100		
Levofloxacin								3 1.2	1 1.6		7 4.4	27 15.2	212 100	
Linezolid						6 2.4	79 34.0	155 96.0	10 100					
Minocycline					123 49.2	7 52.0	4 53.6	7 56.4	30 68.4	59 92.0	20 100			
Penicillin								2 0.8	3 2.0		245 100			
Vancomycin												6 2.4	244 100	

CONCLUSIONS

- There was significant variation observed in VRE rates globally, with higher percentages seen in North America than in Europe, Latin America, the Middle East and Asia/Pacific regions. No E. faecalis, VRE were observed in the Asia/Pacific and Middle East regions although sampling rates were low in these regions.
- Tigecycline exhibited potent in vitro activity against VRE, inhibiting 100% of strains at MIC90 values <=0.25 mcg/mL.
- Tigecycline's MIC90 value of 0.12 mcg/mL against vancomycin-resistant E. faecalis was 16- to 32 fold lower than that of linezolid, ampicillin, or penicillin.
- Tigecycline's MIC90 value of 0.12 mcg/mL against vancomycin-resistant E. faecium was 16-fold lower than that of linezolid, and >64-fold lower than that of any other comparator in this study.
- Tigecycline's potent activity against vancomycin-resistant E. faecalis and E. faecium should make it a useful addition to the list of antimicrobial agents with clinical potential against these resistant strains with limited therapeutic options.