

## Revised Abstract

**Background:** Tigecycline (TIG), the first marketed drug of the glycylicines class of antimicrobials, has been shown to have potent *in vitro* activity against community and hospital acquired staphylococcal and enterococcal pathogens. The Tigecycline Evaluation and Surveillance Trial (TEST) determined the *in vitro* activity against drug-resistant *S. aureus* and *Enterococcus* spp. of TIG and 9 antimicrobials commonly prescribed for serious gram-positive infections: amoxicillin-clavulanic acid (AUG), piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), minocycline (MIN), vancomycin (VAN), ampicillin (AMP), penicillin (P), and imipenem (IMP).

**Methods:** A total of 7,366 clinical isolates (2,822 enterococci; 4,544 *S. aureus*) from North America were identified to the species level at each participating site and confirmed by a central laboratory. Study strains were collected from 259 laboratories in the United States (234) and Canada (25) throughout 2007-2010. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using sponsor supplied broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints were available, with TIG susceptible breakpoints (FDA) defined as  $\leq 0.5$  mcg/ml for *S. aureus* and  $\leq 0.25$  mcg/ml for the enterococci.

**Results:** 20.3% of enterococci were resistant to vancomycin (VRE), and 47.8% of *S. aureus* were resistant to ceftioxin (MRSA). Among the VRE, % resistance rates to other study drugs were LVX 98.8, P 86.0, AMP 86.4, VAN 100, and MIN 22.4. Resistance rates for MRSA were LVX 66, MIN 0.7, and VAN 0. Tigecycline inhibited 98% of all MDR enterococci (resistant to 3 or more drug classes) and 100% of MRSA.

**Conclusions:** TIG retained potent activity against drug-resistant (including MDR) isolates of *S. aureus* and enterococci, inhibiting 100% of all MRSA and >99% of VRE at their defined breakpoints of  $\leq 0.5$  and  $\leq 0.25$  mcg/ml, respectively. TIG should prove to be a useful drug for therapy of infections with these resistant gram-positive pathogens.

## Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) now account for approximately 60% of all serious *S. aureus* infections in the United States [1]. Vancomycin has been the mainstay of treatment for MRSA for over 50 years and has remained remarkably effective. However, the fact that vancomycin is beginning to lose efficacy is showing up clinically as treatment failures and microbiologically as vancomycin heteroresistance. Even newer agents such as linezolid and daptomycin have had resistant isolates identified within a year or two of their introduction. It is clear that additional antimicrobial agents are needed to combat this serious pathogen. Current treatment options against vancomycin resistant *Enterococcus* spp. are largely limited to doxycycline, quinupristin/dalfopristin, and linezolid. Tigecycline has shown potent *in vitro* activity against strains of *Enterococcus* spp. [2-4] and *S. aureus* [3,4].

This study was undertaken to document the *in vitro* activity of tigecycline against a significant number of drug resistant MRSA and VRE *Enterococcus* spp. from a diverse population within North America. This study is part of the larger ongoing global Tigecycline Evaluation and Surveillance Trial (TEST).

## Materials & Methods

- 259 cumulative sites (234 in the United States and 25 in Canada) participated in the TEST program throughout 2007-2010. For this report, 7,366 isolates (2,081 *Enterococcus faecalis*, 741 *E. faecium*, and 4,544 *Staphylococcus aureus*) were identified to the species level and MICs determined at each participating laboratory using sponsor supplied broth microdilution panels. Isolates were derived from blood, respiratory tract, skin and skin structures, bodily fluids, and various other infection sources. Only one isolate per patient was accepted into the study.
- Organism collection, transport, confirmation of organism identification, susceptibility testing, 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.
- Minimum inhibitory concentrations (MICs) were determined by the Clinical and Laboratory Standards Institute (CLSI) recommended broth microdilution testing method using MicroScan (Siemens Medical Solutions Diagnostics, West Sacramento, CA) or Sensititre (TREK Diagnostic Systems, Cleveland, OH) panels [5]. All antimicrobials were supplied by the panel manufacturers.
- MIC interpretive criteria followed published guidelines of the CLSI [6] and the recent United States Food and Drug Administration package insert for tigecycline where applicable [7].
- Quality controls (QC) were performed on each day of testing using appropriate ATCC control strains, following CLSI and manufacturer guidelines. Results were included in the analysis only when corresponding QC results were within the acceptable ranges [2].

## References

- Hidron, A. I., J. R. Edwards, J. Patel, T. C. Horan, D. M. Sievert, D. A. Pollock, and S. K. Fridkin. 2008. *NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007*. Infect. Control Hosp. Epidemiol. 29:996-1011.
- 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.
- Bouchillon, S.K., et al., *In Vitro Activity of Tigecycline Against 3,989 Gram-Negative and Gram-Positive Clinical Isolates from the United States Tigecycline Evaluation and Surveillance Trial (TEST Program; 2004)*. Diagn Microbiol Infect Dis, 2005. 52(3): p. 173-179.
- Hoban, D.J., et al., *In Vitro Activity of Tigecycline Against 6,792 Gram-Negative and Gram-Positive Clinical Isolates from the Global Tigecycline Evaluation and Surveillance Trial (TEST Program; 2004)*. Diagn Microbiol Infect Dis, 2005. 52(3): p. 215-227.
- CLSI, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition*, in Document M7-A8. 2009: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
- CLSI, *Performance Standards for Antimicrobial Susceptibility Testing*, in Document M100-S21. 2011: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
- Tygacil®. 2011. Tigecycline FDA prescribing information. Pfizer, Inc., Collegeville, PA.

## Acknowledgements

We gratefully acknowledge the contributions of the investigators, laboratory personnel, and all members of the Tigecycline Evaluation and Surveillance Trial group. This study was sponsored by Pfizer Inc. IHMA is a clinical research organization that has been contracted by Pfizer Inc. to manage the TEST program.

Table 1. *In vitro* activity of tigecycline and comparators against North American *S. aureus* and *Enterococcus* spp.

Organism <sup>a</sup>	Drug	%Sus <sup>b</sup>	%Int	%Res	MIC (mcg/ml)	
					MIC <sub>50</sub>	MIC <sub>90</sub>
<i>S. aureus</i> (n=4544)	Amox-Clav	52.2	0	47.8	2	> 8
	Ampicillin	12.0	0	88.0	16	> 16
	Ceftriaxone	51.5	0.7	47.8	4	> 64
	Imipenem	20.7	0	79.3	1	4
	Levofloxacin	60.9	2.0	37.0	0.25	32
	Meropenem	52.7	0	47.4	0.25	8
	Minocycline	98.7	0.7	0.6	$\leq 0.25$	0.5
	Penicillin	10.1	0	89.9	> 8	> 8
MRSA (n=2170)	Pip-Tazo	52.2	0	47.8	2	> 16
	Tigecycline	100	0	0	0.12	0.25
	Vancomycin	100	0	0	1	1
	Levofloxacin	31.2	2.7	66.0	4	> 32
<i>Enterococcus</i> spp. (n=2822)	Minocycline	98.3	1.0	0.7	$\leq 0.25$	0.5
	Tigecycline	100	0	0	0.12	0.25
	Vancomycin	100	0	0	1	1
	Ampicillin	77.5	0	22.5	1	> 16
	Levofloxacin	48.4	1.1	50.5	8	> 32
VRE (n=572)	Minocycline	44.9	30.2	24.8	8	> 8
	Penicillin	77.2	0	22.8	2	> 8
	Tigecycline	99.3	0	0.7	0.12	0.25
	Vancomycin	79.2	0.6	20.3	1	> 32
	Ampicillin	13.6	0	86.4	> 16	> 16
Levofloxacin	1.1	0.2	98.8	> 32	> 32	
	Minocycline	56.6	21.0	22.4	4	> 8
	Penicillin	14.0	0	86.0	> 8	> 8
	Tigecycline	99.0	0	1.1	0.06	0.25
Vancomycin	0	0	100	> 32	> 32	

<sup>a</sup>MRSA = methicillin-resistant *S. aureus*; Methicillin phenotype was determined by the susceptibility of *S. aureus* to ceftioxin 30 mcg disk; VRE = vancomycin-resistant enterococci.

<sup>b</sup>Breakpoints as defined by CLSI (M100-S21), 2011. Tigecycline breakpoints defined by FDA (Tygacil®, 2011).

## Results

Table 2. Frequency distribution (n) and cumulative percent inhibited (%) *in vitro* activity of tigecycline and comparative agents against 2,170 methicillin-resistant<sup>a</sup> *S. aureus* from North America.

N / Cum% <sup>b</sup>	MIC (mcg/ml)														
	$\leq 0.008$	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Tigecycline	2	1	16	354	1294	367	136								
Levofloxacin				30	363	202	63	20	59	404	164	224	641		
Minocycline				1.4	18.1	27.4	30.3	31.2	34	52.6	60.1	70.5	100		
Vancomycin						1840	164	71	26	33	36				
						84.8	92.4	95.6	96.8	98.3	100				
						43	888	1169	69						
						2	42.9	96.8	100						

<sup>a</sup>Methicillin phenotype based upon ceftioxin 30 mcg disk results according to CLSI document M100-S21, 2011.

<sup>b</sup>MIC<sub>50</sub> and MIC<sub>90</sub> values in red.

Table 3. Frequency distribution (n) and cumulative percent inhibited (%) *in vitro* activity of tigecycline and comparators against 77 isolates of vancomycin-resistant *Enterococcus faecalis* from North America.

N / Cum% <sup>a</sup>	MIC (mcg/ml)														
	$\leq 0.008$	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Tigecycline	10	19	22	22	2	2									
Ampicillin				13	37.7	66.2	94.8	97.4	100						
Levofloxacin					1	2	19	38	13	1	1	2			
Minocycline							1.3	3.9	28.6	77.9	94.8	96.1	97.4	100	
Penicillin									3	1	0	0	5	68	
Vancomycin									3.9	5.2	0	0	11.7	100	
									21	4	2	3	10	37	
									27.3	32.5	35.1	39	51.9	100	
									1	0	5	26	35	10	
									1.3	0	7.8	41.6	87	100	

<sup>a</sup>MIC<sub>50</sub> and MIC<sub>90</sub> values in red.

Table 4. Frequency distribution (n) and cumulative percent inhibited (%) *in vitro* activity of tigecycline and comparators against 495 isolates of vancomycin-resistant *Enterococcus faecium* from North America.

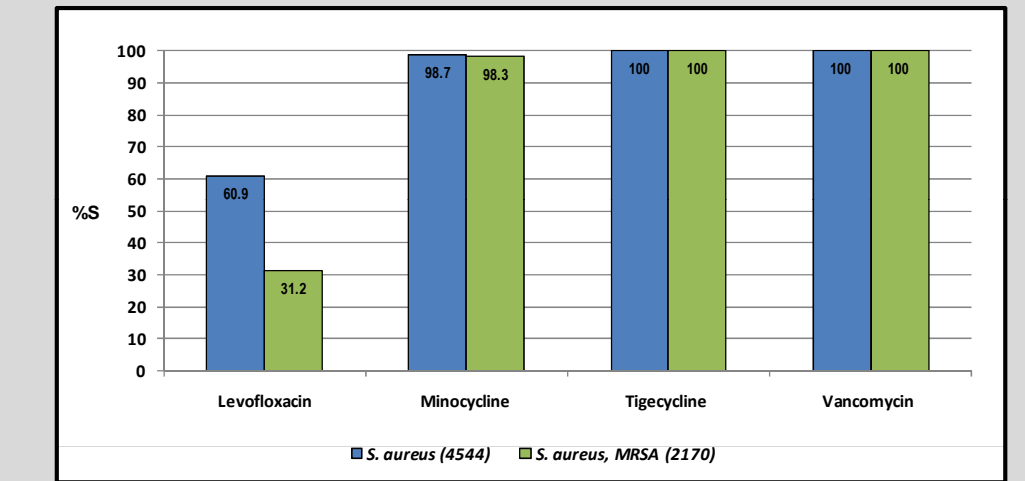
N / Cum% <sup>a</sup>	MIC (mcg/ml)															
	$\leq 0.008$	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64	
Tigecycline	4	135	167	124	63	1	1									
Ampicillin										1	1	0	0	1	492	
Levofloxacin										0.2	0.4	0	0	0.6	100	
Minocycline										0.4	0	0.6	1	2.8	100	
Penicillin										2	0	1	2	9	481	
Vancomycin										0.4	0	0.6	1	2.8	100	
										178	18	11	15	62	211	
										36	39.6	41.8	44.8	57.4	100	
										1	0	1	1	0	492	
										0.2	0	0	0	0.4	0.6	100

<sup>a</sup>MIC<sub>50</sub> and MIC<sub>90</sub> values in red.

## Conclusions

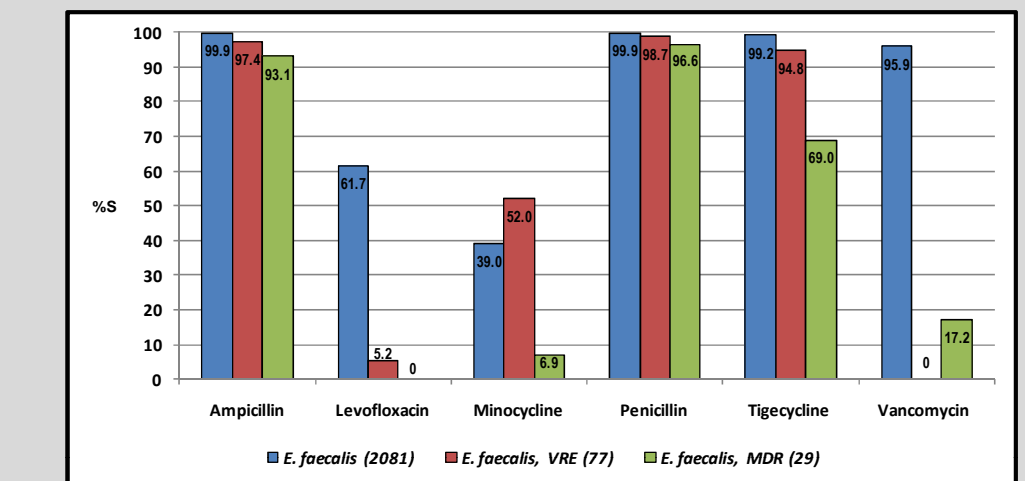
- Tigecycline inhibited 100% of all *S. aureus* and 99.3% *Enterococcus* spp. at the FDA susceptible breakpoints of 0.5 and 0.25 mcg/ml, respectively, without regard to either methicillin- or vancomycin-resistant phenotypes.
- Tigecycline demonstrated equivalent *in vitro* potency to vancomycin against MRSA with 100% susceptibility.
- Tigecycline's MIC<sub>90</sub> of 0.25 mcg/ml against both vancomycin-resistant *E. faecalis* and vancomycin-resistant *E. faecium* was the lowest of all comparator agents in this study.
- The *in vitro* activity of tigecycline in this study suggests that it remains highly active against methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus* spp. and may be an effective treatment option for these increasingly difficult to treat phenotypes.

Figure 1. Susceptibility of North American *S. aureus* and MRSA<sup>a</sup> to tigecycline and comparators.



<sup>a</sup>MRSA=methicillin-resistant *S. aureus*

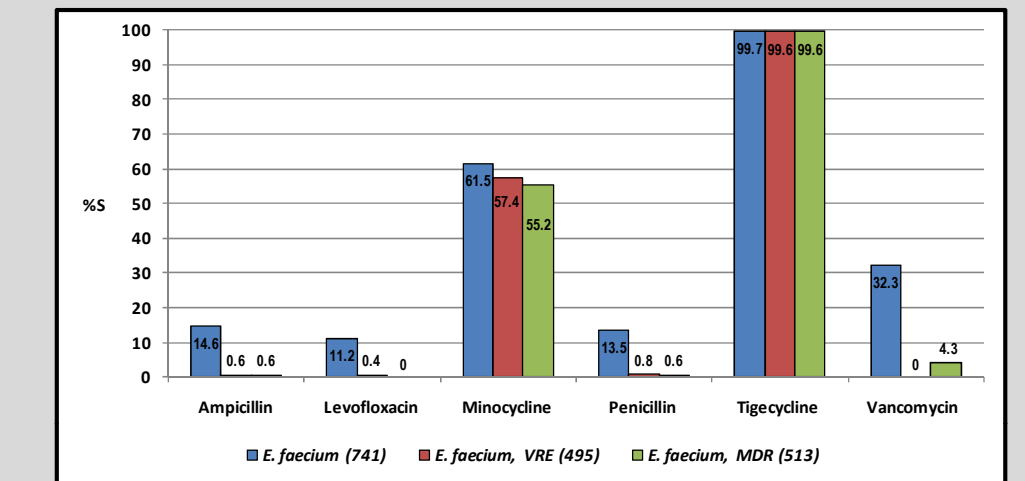
Figure 2. Susceptibility of North American *E. faecalis*, *E. faecalis*, VRE<sup>a</sup> and *E. faecalis*, MDR<sup>b</sup> to tigecycline and comparators.



<sup>a</sup>*E. faecalis*, VRE= vancomycin resistant

<sup>b</sup>*E. faecalis*, MDR= multi-drug resistant, defined as resistant to 3 or more antibiotic classes.

Figure 3. Susceptibility of North American *E. faecium*, *E. faecium*, VRE<sup>a</sup> and *E. faecium*, MDR<sup>b</sup> to tigecycline and comparators.



<sup>a</sup>*E. faecium*, VRE= vancomycin resistant

<sup>b</sup>*E. faecium*, MDR= multi-drug resistant, defined as resistant to 3 or more antibiotic classes.