

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

#FP-C-18

B. Johnson<sup>1</sup>, T. Stevens<sup>1</sup>, S. Bouchillon<sup>1</sup>, J. Johnson<sup>1</sup>, D. Hoban<sup>1</sup>, M. Hackel<sup>1</sup>, M. Person<sup>1</sup>, M. Dowzicky<sup>2</sup>

IHMA, Inc.  
2122 Palmer Dr.  
Schaumburg, IL 60173  
Tel: (847) 303-5003  
Fax: (847) 303-5601  
www.ihmainc.com

<sup>1</sup>International Health Management Associates, Schaumburg, IL, USA  
<sup>2</sup>Wyeth Pharmaceuticals, Collegeville, PA, USA

## REVISED ABSTRACT

**Background:** Resistance of gram positive bacteria continue to be a therapeutic challenge for the clinician. Glycylcyclines are showing the promise of significant activity against many gram-positive pathogens. Tigecycline (GAR-936), a member of this new class of antimicrobials, has shown excellent activity against *Staphylococcus* spp. The in vitro activity of tigecycline as compared with those of 9 comparator agents (ampicillin, amoxicillin-clav, imipenem, ceftriaxone, levofloxacin, minocycline, vancomycin, linezolid, piperacillin-tazobactam) against methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA) from multi-national evaluation centers in the T.E.S.T. **Methods:** A total of 633 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 MICs of tigecycline ranged from 0.03 to 1 for all isolates of *S. aureus*. Tigecycline MIC<sub>50</sub>/MIC<sub>90</sub> of 0.12/0.12 mcg/ml against MSSA 2/8 fold lower than the remaining comparative agents. Tigecycline MIC<sub>50</sub>/MIC<sub>90</sub> of 0.12/0.25 mcg/ml against MRSA was, respectively, 4/2 fold lower than vancomycin, 4/4 fold lower than minocycline and 4/8 fold lower than linezolid. **Conclusion:** The in vitro activity of tigecycline was comparable in all *S. aureus* tested regardless of methicillin phenotype. Tigecycline activity against MRSA was superior to all antimicrobial tested including imipenem, minocycline, linezolid, and vancomycin.

## 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. 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].

While developed to provide activity against tetracycline- and multi-drug-resistant gram-positive pathogens, it has been demonstrated to possess significant broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative microorganisms [1,3-5]. Tigecycline MIC<sub>90</sub> values of  $\leq 0.5$  mcg/ml have been demonstrated against methicillin-resistant *Staphylococcus aureus* (MRSA) [2, 4-6].

Tigecycline resistance is very infrequent and difficult to induce in the laboratory [7, 8] with a selection frequency observed at less than  $10^{-9}$  [2, 3, 7]. Most tetracycline-resistant bacteria with either tetracycline efflux pumps or ribosomal protective features are sensitive to tigecycline [1-4, 6, 9-11]. The pharmacokinetics of parenteral tigecycline is linear with an unusually long half-life of 36 hours and a maximum serum concentration (C<sub>MAX</sub>) of a 300 mg dose infused over 1 hour of 2.8 mcg/ml [12, 13].

This study compared the activity of tigecycline with other agents against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) from hospitals in Europe and North America.

## 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 [14]. Tigecycline tentative breakpoints (in units of mcg/mL) are defined as susceptible  $\leq 2$ ; intermediate = 4; and resistant  $\geq 8$ .

- 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).

## ACKNOWLEDGEMENTS

This study was supported by a grant from Wyeth Pharmaceuticals. We gratefully acknowledge Andre Hsiung for manuscript preparation and the contributions to this study from the following participating institutions: U.S.A., UCLA Medical Center; U.S.A., University of Florida; U.S.A., Jackson Memorial; U.S.A., University of Maryland Medical Center; U.S.A., University of Michigan Medical Center; U.S.A., William Beaumont Hospital; U.S.A., Wake Forest University Baptist Medical Center; U.S.A., Montefiore Medical Center; U.S.A., New York Hospital Queens; U.S.A., Columbia Presbyterian Medical Center; U.S.A., Univ. of Rochester Medical Center; U.S.A., Summa Health System; U.S.A., University Hospitals of Cleveland; U.S.A., RML @ SJMC; U.S.A., Oregon Medical Laboratories; U.S.A., Memorial Hermann Hospital; U.S.A., LDS Hospital; U.S.A., Clarian Health Partners, Inc./Methodist Hospital; Italy, Institute of Micro/Univ. of Ancona Med. School; Canada, Toronto Medical Labs/Mount Sinai Hospital; U.S.A., Marshfield Laboratories; U.S.A., Cleveland Clinic Foundation; U.S.A., Inova Fairfax Hospital; U.S.A., Mercy Health Laboratory; Spain, Hospital General Universitario Gregorio Maranon; United Kingdom, Kings College; China, Peking Union Medical College Hospital; Germany, Inst. of Hygiene - University of Heidelberg; Germany, Institute for Med Microbiology

## REFERENCES

- 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.
- 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.
- 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.
- 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.
- Milatovic, D., et al., Activities of the Glycylcycline Tigecycline (GAR-936) against 1,924 Recent European Clinical Bacterial Isolates. *Antimicrob Agents Chemother*, 2003, 47(1): p. 400-4.
- 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.
- Chopra, I., New developments in tetracycline antibiotics: glycylcyclines and tetracycline efflux pump inhibitors. *Drug Resist Updat*, 2002, 5(3-4): p. 119-25.
- 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.
- 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.
- 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.
- 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.
- 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.
- National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Information Supplement. NCCLS document M100-S12. Wayne, PA, 2002.
- National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Information Supplement. NCCLS document M100-S14. Wayne, PA, 2004.

## RESULTS

Table 1. In Vitro Activity of Tigecycline and Comparator Agents Against 633 Isolates of *Staphylococcus aureus*.

Organism Name	Drug	NCCLS BP*	%S	%I	%R	MIC (mcg/mL)	
						MIC50	MIC90
<i>Staphylococcus aureus</i> (n=633)	Tigecycline	2*	100.0	0.0	0.0	0.12	0.25
	Amox/Clav	4/2	60.9	0.0	39.1	2	>8
	Ampicillin	8	9.3	0.0	90.7	16	>16
	Ceftriaxone	8	53.9	17.7	28.4	8	>64
	Imipenem	4	83.3	2.7	14.1	0.25	>16
	Levofloxacin	2	55.0	7.4	37.6	0.5	>32
	Linezolid	4	100.0	0.0	0.0	2	2
	Minocycline	4	98.3	1.3	0.5	0.25	0.5
	Pip/Tazo	8/4	61.0	0.0	39.0	2	>16
	Vancomycin	4	100.0	0.0	0.0	1	1
<i>Staphylococcus aureus</i> , MSSA (n=344)	Tigecycline	2*	100.0	0.0	0.0	0.12	0.12
	Amox/Clav	4/2	87.6	0.0	12.4	1	8
	Ampicillin	8	16.0	0.0	84.0	8	>16
	Ceftriaxone	8	88.4	3.8	7.8	4	16
	Imipenem	4	95.9	1.5	2.6	0.25	0.5
	Levofloxacin	2	83.7	2.0	14.2	0.12	16
	Linezolid	4	100.0	0.0	0.0	2	4
	Minocycline	4	99.1	0.9	0.0	0.25	0.5
	Pip/Tazo	8/4	89.5	0.0	10.5	1	16
	Vancomycin	4	100.0	0.0	0.0	0.5	1
<i>Staphylococcus aureus</i> , MRSA (n=289)	Tigecycline	2*	100.0	0.0	0.0	0.12	0.25
	Amox/Clav	4/2	26.2	0.0	73.8	8	>8
	Ampicillin	8	1.4	0.0	98.6	>16	>16
	Ceftriaxone	8	12.8	34.3	52.9	64	>64
	Imipenem	4	68.2	4.2	27.7	0.5	>16
	Levofloxacin	2	20.8	13.8	65.4	16	>32
	Linezolid	4	100.0	0.0	0.0	2	2
	Minocycline	4	97.2	1.7	1.0	0.25	1
	Pip/Tazo	8/4	27.0	0.0	73.0	>16	>16
	Vancomycin	4	100.0	0.0	0.0	1	1

\* Breakpoints defined by the NCCLS 2004, document M100-S14; Tigecycline tentative breakpoints (in mcg/mL) defined as susceptible  $\leq 2$ ; intermediate = 4; resistant  $\geq 8$  MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-susceptible *S. aureus*.

Table 2. Frequency Distribution (n) and Cumulative Percent Inhibition (%) at each MIC (mcg/mL) for Tigecycline and Comparative Agents Against 344 Methicillin-sensitive *Staphylococcus aureus*.

	MIC (mcg/mL) - n / Cumulative % - (MIC90)												
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Tigecycline	69	241	28	5	1								
Amox/Clav	20.1	90.1	98.3	99.7	100.0								
Ampicillin	38	31	91	112	31	4	9	28					
Ceftriaxone	26	23	6	13	29	29	30	38	51	99			
Imipenem	7.6	14.2	16.0	19.8	28.2	36.6	45.3	56.4	71.2	100.0			
Levofloxacin	1	12	132	150	9	9	4	3	24				
Linezolid	0.3	3.8	42.2	85.8	88.4	91.0	92.2	93.0	100.0				
Minocycline	116	188	11	7	6	2	5	3	6				
Pip/Tazo	33.7	88.4	91.6	93.6	95.3	95.9	97.4	98.3	100.0				
Vancomycin	53	154	57	14	3	7	7	12	10	11	16		
Linezolid	15.4	60.2	76.7	80.8	81.7	83.7	85.8	89.2	92.2	95.3	100.0		
Minocycline	0.3	15.1	89.2	100.0									
Pip/Tazo	288	39	7	1	6	3							
Vancomycin	83.7	95.1	97.1	97.4	99.1	100.0							
Linezolid	35	120	112	31	6	4	8	28					
Minocycline	10.2	45.1	77.6	86.6	88.4	89.5	91.9	100.0					
Pip/Tazo	2	185	152	5									
Vancomycin	0.6	54.4	98.5	100.0									

Figure 1. In Vitro Activity of Tigecycline and Comparators Against 344 MSSA Showing Cumulative Percent Inhibited (%) at Each MIC (mcg/ml)

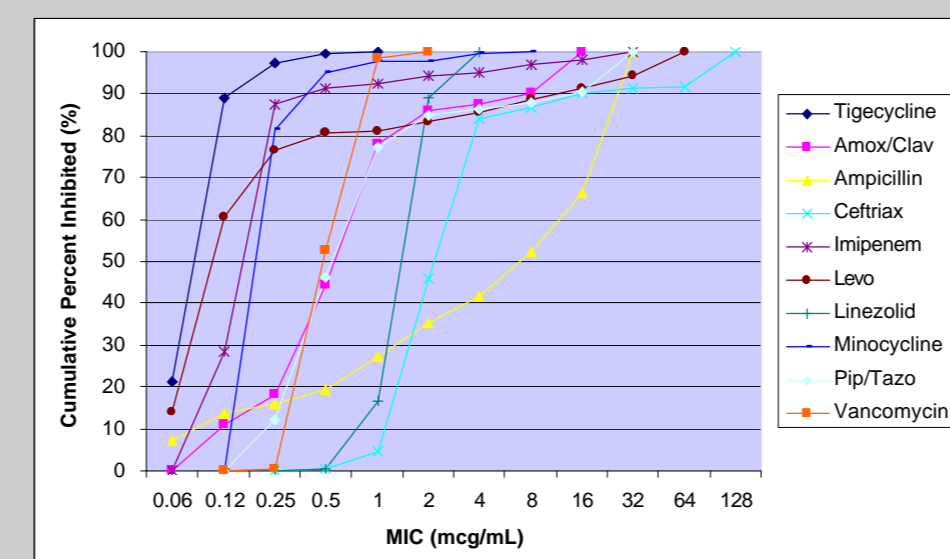
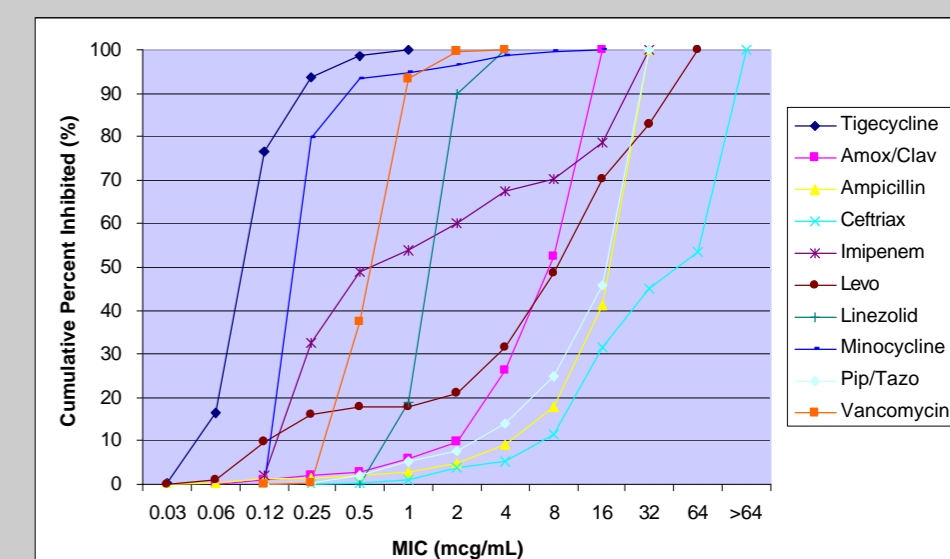


Table 3. Frequency Distribution (n) and Cumulative Percent Inhibition (%) at each MIC (mcg/mL) for Tigecycline and Comparative Agents Against 289 Methicillin-resistant *Staphylococcus aureus*.

	MIC (mcg/mL) - n / Cumulative % - (MIC90)												
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Tigecycline	1.0	41.0	179.0	49.0	15.0	4.0							
Amox/Clav	0.3	14.5	76.5	93.4	98.6	100.0							
Ampicillin	2.0	2.0	1.0	3.0	4.0	16.0	28.0	65.0	168.0				
Ceftriaxone	0.7	1.4	1.7	2.8	4.2	9.7	19.4	41.9	100.0				
Imipenem			1.0	2.0	8.0	6.0	20.0	59.0	40.0	22.0	131.0		
Levofloxacin			0.3	1.0	3.8	5.9	12.8	33.2	47.1	54.7	100.0		
Linezolid			8.0	94.0	45.0	18.0	17.0	15.0	12.0	19.0	61.0		
Minocycline			2.8	35.3	50.9	57.1	63.0	68.2	72.3	78.9	100.0		
Pip/Tazo			5.0	30.0	14.0	4.0	7.0	40.0	40.0	58.0	42.0	49.0	
Vancomycin			1.7	12.1	17.0	18.3	20.8	34.6	48.4	68.5	83.0	100.0	
Linezolid						65.0	202.0	22.0					
Minocycline						22.5	92.4	100.0					
Pip/Tazo						228.0	31.0	3.0	7.0	12.0	5.0	3.0	
Vancomycin						78.5	89.6	90.7	95.1	97.2	99.0	100.0	
Linezolid						1.0	6.0	9.0	10.0	21.0	31.0	61.0	150.0
Minocycline						0.3	2.4	5.5	9.0	16.3	27.0	48.1	100.0
Pip/Tazo						1.0	118.0	156.0	13.0	1.0			
Vancomycin						0.3	41.2	95.2	99.7	100.0			

Figure 2. In Vitro Activity of Tigecycline and Comparators Against 289 MRSA Showing Cumulative Percent Inhibited (%) at Each MIC (mcg/ml)



## CONCLUSIONS

- Tigecycline inhibited the growth of all MSSA and MRSA at a MIC < 1 mcg/ml.
- Tigecycline demonstrates in vitro activity comparable to or better than both vancomycin and linezolid against all MSSA and MRSA.
- Tigecycline demonstrates greater in vitro activity against MSSA and MRSA than levofloxacin, imipenem and the  $\beta$ -lactam antimicrobials.
- Tigecycline appears to be promising agent in the treatment of methicillin sensitive and methicillin resistant *Staphylococcus aureus*.