

# Tigecycline Evaluation Surveillance Trial (T.E.S.T.) - Global In Vitro Antibacterial Activity against 14,705 Gram-positive and Gram-negative Pathogens

#A-095

S. Bouchillon1, T. Stevens1, B. Johnson1, J. Johnson1, D. Hoban1, A. Hsiung1, M. Hackel1, M. Person1, M. Dowzicky2

1International Health Management Associates, Schaumburg, IL, USA  
2Wyeth Pharmaceuticals, Collegeville, PA, USA

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

## REVISED ABSTRACT

**Background:** Tigecycline, 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 amikacin, ampicillin, imipenem, cefepime, ceftazidime, ceftriaxone, levofloxacin, minocycline, and piperacillin-tazobactam against gram-negative strains in addition to linezolid, penicillin, and vancomycin for the gram-positive species. Isolates were collected from 80 hospitals in North America, Europe and Asia throughout 2004. **Methods:** A total of 14,705 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using supplied broth microdilution panels from Dade Behring MicroScan and interpreted according to CLSI guidelines. **Results:** Tigecycline's activity was similar to imipenem's against most *Enterobacteriaceae*. Tigecycline inhibited ESBL producers with an MIC  $\leq 2$  mcg/ml. Although similar to other classes of broad spectrum antimicrobial agents against *Acinetobacter anitratus* and *A. Iwoffii*, tigecycline was especially active against *A. baumannii*, demonstrating the lowest MIC<sub>90</sub> of 1 mcg/ml. Tigecycline had no significant activity against *Pseudomonas aeruginosa*. Tigecycline successfully inhibited *Staphylococcus aureus* with an MIC<sub>90</sub> of 0.25 mcg/ml regardless of methicillin susceptibility phenotype. Similar results were noticed against enterococci with a tigecycline MIC<sub>90</sub> of 0.12 mcg/ml against all strains of enterococci without regard to vancomycin susceptibility. **Conclusion:** Tigecycline's in vitro activity was comparable to or greater than most commonly prescribed antimicrobials against a broad spectrum of aerobic clinical pathogens from a diverse geographical population. The presented data suggest that tigecycline may be an effective therapeutic option against many aerobic gram-positive and gram-negative bacteria, including problematic strains with ESBL, VRE and MRSA resistance phenotypes.

## 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 antibacterial 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 pathogens and has demonstrated significant 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<sup>-9</sup> [3, 5, 7]. With the exception of *Pseudomonas 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 highly effective against multi-resistant *Acinetobacter* spp., particularly *A. baumannii*, which are commonly associated with serious nosocomial infections. Similar activity has been observed against *Enterobacteriaceae*, even extended-spectrum  $\beta$ -lactamase and ampC producing strains [10]. Tigecycline has demonstrated MIC<sub>90</sub> values of  $<0.5$  mcg/ml against methicillin-resistant *S. aureus* (MRSA) and other gram-positive organisms [2, 4-6]. Tigecycline has shown potent activity in animal models infected with selected strains of multi-drug resistant *Enterococcus faecium* and *E. 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 a large, diverse population of clinical isolates collected from hospitals 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.
- Clinical isolates were collected tested between January 2004 - December 2004 from 80 study centers in 19 countries.
- Custom broth microdilution panels were supplied by MicroScan (Dade Behring Inc., 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 followed published guidelines established by the CLSI where applicable [12]. 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 by the local laboratory. Each site tested the isolates using broth microdilution.
- Quality control of broth microdilution panels followed manufacturer's and CLSI guidelines using the following ATCC strains: *E. faecalis* ATCC 29212; *Escherichia coli* ATCC 25922; *Haemophilus influenzae* ATCC 49247; *H. influenzae* ATCC 49766; *S. aureus* ATCC 29213; *Streptococcus pneumoniae* ATCC 49619; and *P. 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).

## 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 Invest 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.
- 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 Update*, 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; Fourteenth Informational Supplement. NCCLS document M100-S14. Wayne, PA, 2004.

## ACKNOWLEDGEMENTS

This study was supported by a grant from Wyeth Pharmaceuticals. We gratefully acknowledge contributions to the T.E.S.T. study from the following participating institutions: Laboratory Sciences of Arizona, U.S.A.; Scottsdale Healthcare - Shea, U.S.A.; UCLA Medical Center, U.S.A.; Shands Hospital at University of Florida, U.S.A.; Jackson Memorial, U.S.A.; Bayfront Medical Center, U.S.A.; Wellstar Kennestone Hospital, U.S.A.; Ochsner Clinic Foundation, U.S.A.; LSU HSC - S, U.S.A.; University of Maryland Medical Center, U.S.A.; University of Michigan Health System, U.S.A.; William Beaumont Hospital, U.S.A.; New Hanover Regional Medical Center, U.S.A.; Wake Forest University Baptist Medical Center, U.S.A.; University Hospital - Newark, U.S.A.; The Valley Hospital - Microbiology, U.S.A.; Albany Medical Center Hospital, U.S.A.; Montefiore Medical Center, U.S.A.; Brookdale 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.; Computec Clinical Laboratories, U.S.A.; RML @ SJMC, U.S.A.; Memorial Hospital, U.S.A.; Oregon Medical Laboratories, U.S.A.; Memorial Hermann Hospital, U.S.A.; Scott & White Memorial Hospital, U.S.A.; LDS Hospital, U.S.A.; Hennepin County Medical Center, U.S.A.; Stanford University Medical Center, U.S.A.; C.H.U. Cote de Nacre, France; Oregon Medical Laboratories, U.S.A.; The Methodist Hospital, U.S.A.; Clarian Health Partners, Inc./Methodist Hospital, U.S.A.; Toronto Medical Labs/Mount Sinai Hospital, Canada; Hopital Cardiologique, France; Institute of Micro/Univ. of Ancona Med. School, Italy; University of Catania, Italy; Charleston Area Medical Center, U.S.A.; Marshfield Laboratories, U.S.A.; University Hospital Geneva, Switzerland; Northside Hospital - Laboratory, U.S.A.; Cleveland Clinic Foundation, U.S.A.; Inova Fairfax Hospital, U.S.A.; Mercy Health Laboratory, U.S.A.; Hartford Hospital, U.S.A.; P.D. Hinduja National Hospital, India; Hospital General Universitario Gregorio Marañon, Spain; Research Institute for Tropical Medicine, Philippines; Kings College, UK; Peking Univ. Medical College Hospital, China; Fletcher Allen Health Care, U.S.A.; Inst. of Hygiene - University of Heidelberg, Germany; P. Stradina Clinical University Hospital, Latvia; Univ. - Hospital of Freiburg, Germany; Fejer Megyei ANTSZ, Hungary; Isala Kliniken - LMM, The Netherlands; National Institute of Public Health, Poland; Institute for Med Microbiology, Germany and Universitätsklinikum Aachen, Germany.

## RESULTS

Table 1. List of countries and number of investigative sites that contributed to T.E.S.T. program

Country	Investigative Sites
Australia	1
Austria	1
Canada	1
China	1
France	2
Germany	1
Hungary	1
India	1
Italy	2
Latvia	1
Pakistan	1
Philippines	1
Poland	1
Singapore	1
Spain	1
Switzerland	1
The Netherlands	1
United Kingdom	2
United States	53
Total	80

Table 2. In vitro activity of tigecycline and comparative agents against 6,279 selected strains of *Enterobacteriaceae*

Organism Name <sup>a</sup>	Drug <sup>b</sup>	%SUS	%INT	%RES	MIC (mcg/ml)	
					MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Enterobacteriaceae</i> (n=6,279)						
Tigecycline	96.8	2.6	0.6	0.5	1	4
Amikacin	98.8	0.6	0.6	2	4	2
Amox-Clav	48.8	7.3	43.9	16	>32	>32
Ampicillin	16.5	6.2	77.3	>32	>32	>32
Cefepime	95.4	1.2	3.2	$\leq 0.5$	2	2
Ceftazidime	86.6	2.7	10.7	$\leq 8$	32	32
Ceftriaxone	88.1	4.6	7.2	0.12	16	16
Imipenem	98.7	0.4	0.9	0.5	1	1
Levofloxacin	87.4	2	10.6	0.06	8	8
Minocycline	85.8	7.3	6.9	2	8	8
Pip-Tazo	90.9	3.9	5.2	1	16	16
<i>E. coli</i> (n=1,883)						
Tigecycline	99.9	0.1	0	0.12	0.25	1
Amikacin	99.3	0.3	0.4	2	4	4
Amox-Clav	75.5	14.6	9.9	8	16	16
Ampicillin	44.7	1	54.3	>32	>32	>32
Cefepime	97.1	0.8	2.1	$\leq 0.5$	$\leq 0.5$	$\leq 0.5$
Ceftazidime	94.4	1.7	3.9	$\leq 8$	$\leq 8$	$\leq 8$
Ceftriaxone	93.9	1.8	4.4	$\leq 0.06$	0.5	0.5
Imipenem	99.7	0	0.3	0.25	0.5	0.5
Levofloxacin	77.2	2.2	20.6	0.03	$\leq 8$	$\leq 8$
Minocycline	83.6	9.6	6.8	1	8	8
Pip-Tazo	96.3	1.4	2.3	1	4	4
<i>K. pneumoniae</i> (n=1,492)						
Tigecycline	94.2	5	0.8	0.5	2	2
Amikacin	97.9	1.2	0.9	2	4	4
Amox-Clav	81.6	7.9	10.5	2	32	32
Ampicillin	3.6	15	81.5	>32	>32	>32
Cefepime	92.7	1.5	5.8	$\leq 0.5$	4	4
Ceftazidime	86.7	1.6	11.7	$\leq 8$	32	32
Ceftriaxone	87.9	3.9	8.2	$\leq 0.06$	32	32
Imipenem	97.3	1.2	1.5	0.5	0.5	0.5
Levofloxacin	88.9	1.4	9.7	0.06	4	4
Minocycline	83	6.6	10.3	2	16	16
Pip-Tazo	91.2	1.7	7.1	2	16	16
<i>K. oxytoca</i> (n=346)						
Tigecycline	98.6	1.4	0	0.25	1	1
Amikacin	99.1	0.3	0.6	2	4	4
Amox-Clav	85.3	4.9	9.8	2	16	16
Ampicillin	4.9	10.1	85	>32	>32	>32
Cefepime	97.1	0.9	2	$\leq 0.5$	1	1
Ceftazidime	93.6	0.9	5.5	$\leq 8$	$\leq 8$	$\leq 8$
Ceftriaxone	92.8	4.9	2.3	$\leq 0.06$	4	4
Imipenem	99.4	0	0.6	0.5	0.5	0.5
Levofloxacin	93.6	3.2	3.2	0.03	1	1
Minocycline	93.1	4.9	2	1	4	4
Pip-Tazo	90.5	0.6	9	1	16	16
ESBL producers ( <i>E. coli</i> )						
Tigecycline	93.4	5.7	0.9	0.5	2	2
Amikacin	90.4	4.4	5.3	4	16	16
Amox-Clav	28.9	40.4	30.7	16	>32	>32
Ampicillin	0.5	0.9	99.2	>32	>32	>32
Cefepime	52.6	9.6	37.7	8	>32	>32
Ceftazidime	21.9	10.1	68	>32	>32	>32
Ceftriaxone	20.6	24.6	54.8	64	>64	>64
Imipenem	92.1	4.8	3.1	0.5	1	1
Levofloxacin	39.5	5.7	54.8	8	>8	>8
Minocycline	67.4	10.6	22	4	>16	>16
Pip-Tazo	70.6	5.3	24.1	8	>128	>128
<i>E. aerogenes</i> (n=484)						
Tigecycline	96.7	2.9	0.4	0.5	1	1
Amikacin	97.7	1.9	0.4	2	4	4
Amox-Clav	6.4	3.5	90.1	>32	>32	>32
Ampicillin	4.1	3.9	91.9	>32	>32	>32
Cefepime	95.5	1.2	3.3	$\leq 0.5$	1	1
Ceftazidime	79.5	5.6	14.9	$\leq 8$	>32	>32
Ceftriaxone	89.3	6	4.8	0.12	16	16
Imipenem	98.8	0	1.2	1	2	2
Levofloxacin	92.8	2.5	4.8	0.06	1	1
Minocycline	90.3	5	4.8	2	4	4
Pip-Tazo	95.4	8.1	3.5	2	4	4
<i>E. agglomerans</i> (n=25)						
Tigecycline	96	4	0	0.25	2	2
Amikacin	100	0	0	2	8	8
Amox-Clav	48	4	48	16	>32	>32
Ampicillin	20	12	68	>32	>32	>32

<sup>a</sup> Only species with n >20 are represented.  
<sup>b</sup> Breakpoints as defined by CLSI where available (M100-S14), 2004. Tigecycline breakpoints defined as: susceptible  $\leq 2$ ; intermediate = 4; and resistant  $\geq 8$ .

Table 3. In vitro activity of tigecycline and comparative agents selected against 2,586 *Acinetobacter* spp and *Pseudomonas aeruginosa*

Organism Name <sup>a</sup>	Drug <sup>b</sup>	%SUS	%INT	%RES	MIC (mcg/ml)	
					MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Acinetobacter</i> spp (n=1,067)						
Tigecycline	98.9	1.1	0	0.25	1	1
Amikacin	81.5	6.3	12.2	4	64	64
Amox-Clav	na	na	na	>32	>32	>32
Ampicillin	na	na	na	>32	>32	>32
Cefepime	51.4	14.9	33.7	8	>32	>32
Ceftazidime	51	5.6	43.5	$\leq 8$	>32	>32
Ceftriaxone	32.8	22.1	45.1	32	>64	>64
Minocycline	86.1	4.8	9.1	0.5	8	8
Levofloxacin	53	7.5	39.5	2	>8	>8
Minocycline	90.5	6.9	2.5	$\leq 0.5$	4	4
Pip-Tazo	72.9	0	27.1	8	>128	>128
<i>Acinetobacter anitratus</i> (n=21)						
Tigecycline	100	0	0	0.12	0.25	0.25
Amikacin	95.2	0	4.8	2	4	4
Amox-Clav	na	na	na	16	32	32
Ampicillin	na	na	na	16	32	32
Cefepime	95.2	4.8	0	4	8	8
Ceftazidime	90.5	4.8	4.8	$\leq 8$	$\leq 8$	$\leq 8$
Ceftriaxone	57.1	38.1	4.8	8	16	16
Imipenem	100	0	0	0.5	0.5	0.5
Levofloxacin	100	0	0	0.12	0.5	0.5
Minocycline	100	0	0	$\leq 0.5$	$\leq 0.5$	$\leq 0.5$
Pip-Tazo	100	0	0	1	8	8
<i>Acinetobacter baumannii</i> (n=940)						
Tigecycline	98.7	1.3	0	0.25	1	1
Amikacin	80	6.9	13.1	4	64	64
Amox-Clav	na	na	na	32	>32	>32
Ampicillin	na	na	na	>32	>32	>32
Cefepime	47.3	16.2	36.5	16	>32	>32
Ceftazidime	47.6	5.4	46.9	16	>32	>32
Ceftriaxone	28.2	22.6	49.3	32	>64	>64
Imipenem	84.6	5.1	10.3	0.5	16	16
Levofloxacin	48.9	8.2	42.9	4	>8	>8
Minocycline	89.4	7.8	2.9	$\leq 0.5$	8	8
Pip-Tazo	71	0	29	16	>128	>128
<i>Acinetobacter Iwoffii</i> (n=69)						
Tigecycline	100	0	0	0.06	0.25	0.25
Amikacin	100	0	0	1	2	2
Amox-Clav	na	na	na	2	16	16
Ampicillin	na	na	na	4	>32	>32
Cefepime	95.7	2.9	1.4	1	8	8
Ceftazidime	84.1	7.2	8.7	$\leq 8$	16	16
Ceftriaxone	88.4	10.1	1.4	4	16	16
Imipenem	100	0	0	0.25	0.5	0.5
Levofloxacin	95.7	4.3	0	0.06	0.5	0.5