Tigecycline Evaluation Surveillance Trial (T.E.S.T.) - United States In Vitro Antibacterial Activity Against Selected Species of Glucose Non-fermenting Organisms

S. Bouchillon[1], R. Badal[1], B. Johnson[1], J. Johnson[1], D. Hoban[1], M. Hackel[1], M. Dowzicky[2]

[1]International Health Management Associates, Schaumburg, IL, USA
[2]Wyeth 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: Glucose non-fermenting gram-negative rods are known to be highly resistant in hospital settings and have always been a challenge for clinicians. The degree or type of resistance may be due to several sophisticated mechanisms, inactivating most classes of antimicrobials that are available for treatment. Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most species of *Enterobacteriaceae* and selected species of non-fermenters, as well as gram-positives, atypicals, and anaerobes. 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 members of *Acinetobacter* spp. and *Pseudomonas aeruginosa* collected from 137 hospitals in the United States. **Methods:** A total of 5,300 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Isolates were collected from 2004 through 2006. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using broth microdilution panels and interpreted according to CLSI guidelines. **Results:** Tigecycline's activity against *P. aeruginosa* showed an MIC_{90} of >16 mcg/ml. Towards *A. baumannii* (n=1,843), against which cephalosporins were ineffective, tigecycline showed the lowest $MIC_{50/90}$ of 0.5/1 mcg/ml, outperforming amikacin ($MIC_{50/90}$ =4/32), imipenem ($MIC_{50/90}$ =0.5/8), and minocycline ($MIC_{50/90}$ ≤0.5/8). Similar findings were observed in other species of *Acinetobacter*. **Conclusion:** The presented data suggest that tigecycline may be an effective and reliable therapeutic option against strains of *Acinetobacter* spp., including multi-drug resistant strains regardless of degree or type of resistance.

INTRODUCTION

Glucose non-fermenting gram-negative rods are known to be highly resistant in hospital settings and have always been a challenge for clinicians. The degree or type of resistance may be due to several sophisticated mechanisms, inactivating most classes of antimicrobials that are available for treatment.

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 tigecycline 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 minimum inhibitory concentrations for the 90th percentile inhibited 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]. This study was undertaken to document the in vitro activity of tigecycline against selected species of glucose non-fermenting organisms collected in the United States. 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 (no more than 25% of all isolates), skin, wound, body fluids, and other defined sources. Only one isolate per patient was accepted into the study.
- For this study, 5,300 clinical isolates were collected and tested from 2004 to 2006 from 137 hospitals in the United States. 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 was coordinated by Laboratories International for Microbiology Studies (LIMS), a division of International Health Management Associates, Inc. located in Schaumburg, Illinois, 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.
- Minimum inhibitory concentrations (MICs) were determined by the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) recommended broth microdilution testing method [7]. Tigecycline was supplied by Wyeth Pharmaceuticals (Collegeville, Pennsylvania, USA). All other agents were supplied by the panel manufacturer, MicroScan (Dade Behring Inc., Sacramento, California, USA). The following antimicrobial agents were included on the panels with their dilution ranges (expressed in mcg/ml): amikacin (0.5-64); cefepime (0.5-32); ceftriaxone (0.06-64); ceftazidime (8-32); imipenem (0.06-16); levofloxacin (0.008-8); meropenem (0.06-16); minocycline (0.5-16); tigecycline (0.008-16); and piperacillin/tazobactam (0.06/4-128/4). MIC interpretive criteria followed published guidelines established by the CLSI [8] and recent US Food and Drug Administration packaging insert for tigecycline [9], where applicable.
- Quality controls (QC) were performed by each testing site on each day of testing using the corresponding ATCC control strains: *E. coli* ATCC 25922; *E. coli* ATCC 35218; *Klebsiella pneumoniae* ATCC 700603; and *Pseudomonas aeruginosa* ATCC 27853. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI (2006) guidelines [8].

REFERENCES

1. Hoellman, D.B., et al., *Antipneumococcal activities of GAR-936 (a new glycylcycline) compared to those of nine other agents against penicillin-susceptible and -resistant pneumococci*. Antimicrob Agents Chemother, 2000. 44(4): p. 1085-8.
2. Labthavikul, P., P.J. Petersen, and P.A. Bradford, *In vitro activity of tigecycline against Staphylococcus epidermidis growing in an adherent-cell biofilm model*. Antimicrob Agents Chemother, 2003. 47(12): p. 3967-9.
3. 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.
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. 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.
6. Rupp, M.E. and P.D. Fey, *Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment*. Drugs, 2003. 63(4): p. 353-65.
7. *CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Sixth Edition, in Document M7-A6. 2005*: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
8. *CLSI, Performance Standards for Antimicrobial Susceptibility Testing, in Document M100-S16. 2006*: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
9. Tygacil, *Product Insert. 2005*: Wyeth Pharmaceuticals, Inc., Philadelphia, PA, USA.

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RESULTS

The results are listed in the following tables.

Table 1. In vitro activity of tigecycline and comparative agents against glucose non-fermenting organisms.

Organism Name	Drug	n	%SUS[a]	%INT[a]	%RES[a]	MIC (mcg/ml) MIC_{50}	MIC_{90}
Acinetobacter spp.	Tigecycline	2,035	na	na	na	0.5	1
	Amikacin	2,035	85.6	6.7	7.7	4	32
	Cefepime	2,035	51.2	14.5	34.3	8	>32
	Ceftazidime	2,035	51.5	6.3	42.1	≤8	>32
	Ceftriaxone	2,035	32.9	25.4	41.7	32	>64
	Imipenem	1,947	89.1	1.6	9.2	0.5	8
	Levofloxacin	2,035	52.8	5.5	41.7	1	>8
	Meropenem	88	97.7	1.1	1.1	1	4
	Minocycline	2,035	89.4	7.7	2.9	≤0.5	8
	PipTazo	2,035	61.4	17.1	21.6	4	>128
Acinetobacter spp. (multi-drug resistant)	Tigecycline	509	na	na	na	1	2
	Amikacin	509	59.1	15.3	25.5	16	>64
	Cefepime	509	1.6	16.9	81.5	32	>32
	Ceftazidime	509	2.4	2.4	95.3	>32	>32
	Ceftriaxone	509	0.4	3.9	95.7	>64	>64
	Imipenem	491	61.3	4.1	34.6	4	>16
	Levofloxacin	509	1.2	1.6	97.2	>8	>8
	Meropenem	18	94.4	0	5.6	2	4
	Minocycline	509	72.1	17.7	10.2	2	16
	PipTazo	509	4.3	16.5	79.2	128	>128
A. baumannii [b]	Tigecycline	1,843	na	na	na	0.5	1
	Amikacin	1,843	84.6	7.1	8.2	4	32
	Cefepime	1,843	48.1	15.5	36.4	16	>32
	Ceftazidime	1,843	49.3	6.1	44.6	16	>32
	Ceftriaxone	1,843	29.5	26.1	44.4	32	>64
	Imipenem	1,759	88.3	1.8	9.8	0.5	8
	Levofloxacin	1,843	49.4	5.8	44.8	4	>8
	Meropenem	84	97.6	1.2	1.2	1	4
	Minocycline	1,843	88.7	8.2	3.1	≤0.5	8
	PipTazo	1,843	58.8	18.2	23	8	>128
A. lwoffii [c]	Tigecycline	135	99.3	0.7	0	0.12	0.5
	Amikacin	135	97.8	1.5	0.7	1	4
	Cefepime	135	90.4	3	6.7	1	8
	Ceftazidime	135	77.8	8.9	13.3	≤8	32
	Ceftriaxone	135	74.8	19.3	5.9	4	32
	Imipenem	133	97.7	0	2.3	0.25	0.5
	Levofloxacin	135	94.1	3	3	0.12	1
	Meropenem d	2	100	0	0	-	-
	Minocycline	135	99.3	0	0.7	≤0.5	1
	PipTazo	135	94.1	1.5	4.4	≤0.06	8
A. anitratus [e]	Tigecycline	19	89.5	10.5	0	1	4
	Amikacin	19	73.7	15.8	10.5	8	>64
	Cefepime	19	31.6	10.5	57.9	32	>32
	Ceftazidime	19	36.8	0	63.2	>32	>32
	Ceftriaxone	19	15.8	15.8	68.4	>64	>64
	Imipenem	19	78.9	0	21.1	1	>16
	Levofloxacin	19	31.6	5.3	63.2	8	>8
	Minocycline	19	68.4	26.3	5.3	1	8
	PipTazo	19	31.6	31.6	36.8	64	>128
A. calcoaceticus [f]	Tigecycline	15	100	0	0	0.25	2
	Amikacin	15	93.3	0	6.7	2	8
	Cefepime	15	60	13.3	26.7	8	>32
	Ceftazidime	15	66.7	13.3	20	≤8	>32
	Ceftriaxone	15	40	33.3	26.7	16	>64
	Imipenem	15	100	0	0	0.5	1
	Levofloxacin	15	66.7	0	33.3	0.12	>8
	Minocycline	15	93.3	6.7	0	≤0.5	2
	PipTazo	15	80	6.7	13.3	4	128
P. aeruginosa	Tigecycline	3,265	6.2	13.5	80.3	8	>16
	Amikacin	3,265	97.3	1.2	1.5	4	8
	Cefepime	3,265	78.2	12.4	9.4	4	16
	Ceftazidime	3,265	82.8	5.6	11.6	≤8	32
	Ceftriaxone	3,265	17.7	33.6	48.6	32	>64
	Imipenem	3,057	84.6	7.8	7.6	1	8
	Levofloxacin	3,265	63.1	7.3	29.6	1	>8
	Meropenem	208	79.3	9.1	11.5	1	16
	Minocycline	3,265	4.9	15.6	79.5	>16	>16
	PipTazo	3,265	90.3	0	9.7	4	64

[a] Interpretive criteria as defined by CLSI, M100-S16 (2006), where available; tigecycline susceptibility breakpoints are according to FDA package insert (Tygacil®, 2005), where available [9]; na = no interpretive breakpoints available at this time.
[b] Includes 495 multi-drug resistant strains with tigecycline MIC_{90} of 2 mcg/ml (94.9% susceptible).
[c] Includes 4 multi-drug resistant strains with tigecycline MICs ≤1 mcg/ml (all susceptible).
[d] No MIC_{50} and MIC_{90} calculated if n<10; %SUS, %INT, and % RES may not be statistically significant when n's are small.
[e] Includes 7 multi-drug resistant strains with tigecycline MICs ≤4 mcg/ml.
[f] Includes 3 multi-drug resistant strains with tigecycline MICs ≤2 mcg/ml (all susceptible).

Table 2. In vitro activity of tigecycline and comparators against *Acinetobacter* spp. (n=2,035) showing frequency distribution (n), cumulative percent inhibited (%) at each MIC (mcg/ml), and MIC_{90} (highlighted in yellow).

MIC / n / %	≤0.008	0.015	0.03	≤0.06	0.06	≤0.12	0.12	0.25	≤0.5	0.5	1	2	4	≤8	8	>8	16	>16	32	>32	64	>64	128	>128
Tigecycline	5	3	28		243		448	290		399	437	139	37		6									
	0.2	0.4	1.8		13.7		35.7	50		69.6	91.1	97.9	99.7		100									
Amikacin									62		209	624	554		164		129		137		54	102		
									3		13.3	44	71.2		79.3		85.6		92.3		95	100		
Cefepime									120		150	288	262		222		295		327	371				
									5.9		13.3	27.4	40.3		51.2		65.7		81.8	100				
Ceftazidime														1049			129		120	737				
														51.5			57.9		63.8	100				
Ceftriaxone				22			3	6		8	21	67	171		372		343		174		116	732		
				1.1			1.2	1.5		1.9	2.9	6.2	14.6		32.9		49.8		58.3		64	100		
Imipenem				13			36	596		554	223	151	162		32		74	106						
				0.7			2.5	33.1		61.6	73	80.8	89.1		90.8		94.6	100						
Levofloxacin	6	7	43		343		410	150		40	38	38	112		288	560								
	0.3	0.6	2.8		19.6		39.8	47.1		49.1	51	52.8	58.3		72.5	100								
Meropenem				3			3	7		26	19	19	9		1			1						
				3.4			6.8	14.8		44.3	65.9	87.5	97.7		98.9			100						
Minocycline									1230		265	195	129		157		50	9						
									60.4		73.5	83	89.4		97.1		99.6	100						
PipTazo				496			87	91		81	79	90	111		106		108		144		203		213	226
				24.4			28.6	33.1		37.1	41	45.4	50.9		56.1		61.4		68.5		78.4		88.9	100

Table 3. In vitro activity of tigecycline and comparators against multi-drug resistant *Acinetobacter* spp. (n=509) showing frequency distribution (n), cumulative percent inhibited (%) at each MIC (mcg/ml), and MIC_{90} (highlighted in yellow).

MIC / n / %	≤0.06	0.12	0.25	≤0.5	0.5	1	2	4	≤8	8	>8	16	>16	32	>32	64	>64	128	>128
Tigecycline		2	30		116	251	84	24		2									
		0.4	6.3		29.1	78.4	94.9	99.6		100									
Amikacin				1		7	56	111		58		68		78		43	87		
				0.2		1.6	12.6	34.4		45.8		59.1		74.5		82.9	100		
Cefepime				1			1	2		4		86		170	245				
				0.2			0.4	0.8		1.6		18.5		51.9	100				
Ceftazidime									12			12		52	433				
									2.4			4.7		14.9	100				
Ceftriaxone	1									1		10		10		36	451		
	0.2									0.4		2.4		4.3		11.4	100		
Imipenem			5		33	88	76	99		20		70	100						
			1		7.7	25.7	41.1	61.3		65.4		79.6	100						
Levofloxacin			2				4	8		130	365								
			0.4				1.2	2.8		28.3	100								
Meropenem						1	12	4					1						
						5.6	72.2	94.4					100						
Minocycline				103		133	78	53		90		44	8						
				20.2		46.4	61.7	72.1		89.8		98.4	100						
PipTazo	3	1			3	2		5		1		7		34		50		194	209
	0.6	0.8			1.4	1.8		2.8		2.9		4.3		11		20.8		58.9	100

CONCLUSIONS

- Tigecycline's MIC_{90} of 1 mcg/ml against *Acinetobacter* spp. was at least four-fold lower than all other comparators. Although there is not yet a tigecycline interpretive breakpoint for *Acinetobacter*, it inhibited 97.9% of all *Acinetobacter* spp. strains at the *Enterobacteriaceae* susceptible breakpoint of 2 mcg/ml. Only meropenem showed comparable activity.
- Against multi-drug resistant *Acinetobacter* spp. tigecycline showed no significant increase in MIC_{90} (2 mcg/ml), which was 2-fold lower than meropenem's and at least 8-fold lower than for all other comparators. Tigecycline inhibited 94.9% of all multi-drug resistant *Acinetobacter* spp. (again, using the *Enterobacteriaceae* interpretive breakpoint of 2 mcg/ml in the absence of *Acinetobacter* breakpoints), more than any other antimicrobial tested. Only meropenem showed comparable activity.
- Tigecycline's limited activity against *P. aeruginosa* is similar to that of other tetracyclines and their analog derivatives.
- These data suggest that tigecycline may be an effective and reliable therapeutic option against *Acinetobacter* spp., including multi-drug resistant strains regardless of degree or type of resistance.