

REVISED ABSTRACT

Background: Tigecycline, the first member of the glycolcyclines, and has been shown to have potent activity against most Gram-negative and Gram-positive pathogens. The T.E.S.T. program reports the in vitro activity of tigecycline and comparator antimicrobials against nosocomial gram-positive and gram-negative species from South America. **Methods:** A total of 1,267 clinical isolates were collected from 8 hospitals across South America. MICs were determined by broth microdilution according to CLSI guidelines. **Results:** Tigecycline demonstrated a MIC₉₀ value of 1 mcg/mL against all organisms, Gram-positive and Gram-negative (excluding *Pseudomonas*). Tigecycline's activity was similar to imipenem against *Enterobacteriaceae*. It inhibited multi-resistant ESBL-producers with MICs equal to or less than 2 mcg/ml. Although similar to other classes of broad spectrum antimicrobials against non-fermenters, tigecycline was especially active against *Acinetobacter spp.*, presenting the lowest MIC₉₀ of 1 mcg/ml, but was not active against *Pseudomonas*. Tigecycline successfully inhibited 99% *S. aureus* with a MIC₉₀ of 0.5 mcg/ml regardless of sensitivity or resistance to methicillin. The same phenomenon was noticed against enterococci, with tigecycline's MIC₉₀ at 0.25 mcg/ml for both vancomycin-resistant and -susceptible strains. **Conclusion:** Tigecycline in vitro activity was comparable to, and in some instances greater than, most commonly prescribed broad spectrum antimicrobials. Tigecycline's activity was retained even against strains resistant to other antimicrobials, such as ESBL-producers, multi-resistant *Acinetobacter spp.*, methicillin-resistant *S. aureus*, vancomycin-resistant enterococci, and penicillin-resistant *S. pneumoniae*.

INTRODUCTION

Tigecycline (formerly GAR-936) is a member of a new class of antimicrobial agents, the glycolcyclines. 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 glycolcyclines 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 restore 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 significant numbers of clinical pathogens collected in 10 laboratories from South America. 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. Clinical isolates were collected and tested between 2004 to 2006 from 10 study centers in South America. 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 criteria.
- Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [7]. 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 were included on the panels with their dilution ranges (expressed in mcg/ml): amikacin (0.5-64); amoxicillin/clavulanic acid (0.12/0.06-32/16); ampicillin (0.5-32, gram-negative panel, and 0.06-16, gram-positive panel); cefepime (0.5-32); ceftazidime (0.06-64); ceftazidime (8-32); 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 [8] and the recent US Food and Drug Administration package insert for tigecycline [9], where applicable.
- Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were screened for ESBL activity when MIC results for ceftazidime were >1 mcg/ml using broth microdilution panels. ESBL activity was confirmed using the CLSI (2006) phenotypic confirmatory disk test (Oxoid, Ogdensburg, NY, USA) on Mueller-Hinton agar (Remel Inc., Lenexa, KS, USA) according to CLSI (2006) guidelines. ESBL presence was confirmed by testing the following antibiotic disks: cefotaxime (30-mcg), cefotaxime/clavulanic acid (30/10-mcg), ceftazidime (30-mcg), and ceftazidime/clavulanic acid (30/10-mcg). Antimicrobial disks were manufactured by Oxoid, Inc. (Ogdensburg, NY, USA). Mueller-Hinton agar used in testing was manufactured by Remel, Inc. (Lenexa, KS, USA). An organism was interpreted as containing an ESBL if there was an increase of >5 mm in the inhibition zone of the combination disk when compared to that of the cephalosporin alone. *K. pneumoniae* ATCC 700793 was used to QC the ESBL confirmation test. *K. pneumoniae* ATCC 700603 was used to QC the ESBL confirmation tests.
- 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; *H. influenzae* ATCC 49766; *H. influenzae* ATCC 49247; *S. aureus* ATCC 29213; *Pseudomonas aeruginosa* ATCC 27853; *Enterococcus faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI (2006) guidelines [8].

REFERENCES

- Hoeffman, D.B., et al., Antipneumococcal activities of GAR-936 (a new glycolcycline) compared to those of nine other agents against penicillin-susceptible and -resistant pneumococci. *Antimicrob Agents Chemother*, 2000, 44(4): p. 1085-9.
- Lattavaki, P., P.J. Pieterse, 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.
- Projan, S.J., Preclinical pharmacology of GAR-936, a novel glycolcycline antibacterial agent. *Pharmacotherapy*, 2000, 20(9 Pt 2): p. 2195-223S; discussion 224S-228S.
- Giles, A.C. and R.N. Jones, Antimicrobial activity and spectrum of the new glycolcycline, GAR-936 tested against 1,203 recent clinical bacterial isolates. *Diagn Microbiol Infect Dis*, 2000, 36(1): p. 19-36.
- 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. 177-8.
- 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.
- CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Sixth Edition, in Document M7-A6. 2006. 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-S15. 2005. Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
- Tygard, Product insert. 2005. Wyeth Pharmaceuticals, Inc., Philadelphia, PA, USA.

ACKNOWLEDGEMENTS

We gratefully acknowledge the contributions of the investigators, laboratory personnel, and all members of the Tigecycline Evaluation Study Trials program group. This study was sponsored by a grant from Wyeth Pharmaceuticals.

RESULTS

Table 1. In vitro activity of tigecycline and comparator agents against *Enterobacteriaceae*.

Organism	Drug	N=	%Sus	%Int	%Res	MIC ₅₀	MIC ₉₀	LOW	HIGH	
<i>Enterobacter spp</i>	Amikacin	139	87.8	6.5	5.8	2	32	<0.5	>64	
	AmoxClav	139	2.9	0.7	96.4	>32	>32	1	>32	
	Ampicillin	139	0	6.5	93.5	>32	>32	16	>32	
	Cefepime	139	77	8.6	14.4	<0.5	32	<0.5	>32	
	Ceftazidime	139	59.7	5.8	34.5	<8	>32	<8	>32	
	Ceftriaxone	139	61.2	10.8	28.1	1	>64	<0.06	>64	
	Imipenem	136	100	0	0	0.5	1	0.25	4	
	Levofloxacin	139	80.6	5.8	13.7	0.06	>8	<0.008	>8	
	Meropenem	3	100	0	0	<0.06	<0.06	<0.06	<0.06	
	Minocycline	139	79.9	6.5	13.7	4	16	<0.5	>16	
	PipTazo	139	72.7	14.4	12.9	2	128	0.5	>128	
	Tigecycline	139	95	4.3	0.7	0.5	2	0.12	8	
	<i>Escherichia coli</i>	Amikacin	168	98.8	0.6	0.6	2	8	<0.5	64
		AmoxClav	168	69.6	15.5	14.9	8	32	0.5	>32
Ampicillin		168	37.5	0	62.5	>32	>32	<0.5	>32	
Cefepime		168	91.1	2.4	6.5	<0.5	4	<0.5	>32	
Ceftazidime		168	94.6	3	2.4	<8	<8	<8	>32	
Ceftriaxone		168	89.9	0.6	9.5	<0.06	16	<0.06	>64	
Imipenem		168	100	0	0	0.5	0.5	0.25	4	
Levofloxacin		168	75	2.4	22.6	0.03	8	<0.008	>8	
Minocycline		168	79.2	12.5	8.3	1	8	<0.5	>16	
PipTazo		168	95.2	1.8	3	1	4	0.25	>128	
Tigecycline		168	100	0	0	0.12	0.25	<0.008	0.5	
<i>Klebsiella spp</i>		Amikacin	165	93.9	4.2	1.8	2	16	1	>64
		AmoxClav	165	52.7	6.7	40.6	8	>32	0.5	>32
		Ampicillin	165	0	9.7	90.3	>32	>32	16	>32
	Cefepime	165	66.7	5.5	27.9	<0.5	>32	<0.5	>32	
	Ceftazidime	165	66.1	6.1	27.9	<8	>32	<8	>32	
	Ceftriaxone	165	59.4	4.8	35.8	0.12	>64	<0.06	>64	
	Imipenem	162	100	0	0	0.5	0.5	0.12	4	
	Levofloxacin	165	77.6	4.2	18.2	0.06	>8	<0.008	>8	
	Meropenem	3	100	0	0	<0.06	0.12	<0.06	0.12	
	Minocycline	165	83.6	7.9	8.5	2	8	<0.5	>16	
	PipTazo	165	66.1	9.7	24.2	2	>128	0.5	>128	
	Tigecycline	165	96.4	3	0.6	0.5	1	0.12	8	
	<i>Serratia spp</i>	Amikacin	62	88.7	11.3	0	4	32	1	32
		AmoxClav	62	3.2	4.8	91.9	>32	>32	8	>32
Ampicillin		62	0	6.5	93.5	>32	>32	16	>32	
Cefepime		62	88.7	3.2	8.1	<0.5	16	<0.5	>32	
Ceftazidime		62	87.1	4.8	8.1	<8	16	<8	>32	
Ceftriaxone		62	87.1	1.6	11.3	0.25	>64	<0.06	>64	
Imipenem		61	100	0	0	0.5	1	0.5	4	
Levofloxacin		62	95.2	3.2	1.6	0.12	2	<0.008	>8	
Meropenem		1	100	0	0	<0.06	<0.06	<0.06	<0.06	
Minocycline		62	93.5	4.8	1.6	2	4	<0.5	16	
PipTazo		62	87.1	11.3	1.6	1	32	0.5	>128	
Tigecycline		62	98.4	1.6	0	0.5	2	0.25	4	
All ESBLs		Amikacin	72	87.5	9.7	2.8	8	32	1	>64
		AmoxClav	72	8.3	16.7	75	>32	>32	2	>32
	Ampicillin	72	0	0	100	>32	>32	32	>32	
	Cefepime	72	20.8	11.1	68.1	>32	>32	1	>32	
	Ceftazidime	72	20.8	16.7	62.5	>32	>32	<8	>32	
	Ceftriaxone	72	5.6	9.7	84.7	>64	>64	2	>64	
	Imipenem	70	100	0	0	0.5	0.5	0.12	2	
	Levofloxacin	72	48.6	4.2	47.2	4	>8	0.03	>8	
	Meropenem	2	100	0	0	<0.06	0.12	<0.06	0.12	
	Minocycline	72	73.6	16.7	9.7	2	8	<0.5	>16	
	PipTazo	72	40.3	18.1	41.7	64	>128	1	>128	
	Tigecycline	72	94.4	5.6	0	0.5	2	0.12	4	

Table 2. In vitro activity of tigecycline and comparator agents against *Acinetobacter spp.* and *Pseudomonas aeruginosa*.

Organism	Drug	N=	%Sus	%Int	%Res	MIC ₅₀	MIC ₉₀	LOW	HIGH
<i>Acinetobacter spp</i>	Amikacin	95	12.6	17.9	69.5	64	>64	1	>64
	Cefepime	95	10.5	25.3	64.2	32	>32	<0.5	>32
	Ceftazidime	95	7.4	4.2	88.4	>32	>32	<8	>32
	Ceftriaxone	95	1.1	6.3	92.6	>64	>64	8	>64
	Imipenem	95	45.3	8.4	46.3	8	>16	0.25	>16
	Levofloxacin	95	6.3	23.2	70.5	8	>8	0.03	>8
	Minocycline	95	98.9	1.1	0	<0.5	2	<0.5	8
	PipTazo	95	11.6	20	68.4	>128	>128	<0.06	>128
	Tigecycline	95	na	na	na	0.5	1	0.06	4
	<i>Pseudomonas aeruginosa</i>	Amikacin	149	73.2	7.4	19.5	4	64	<0.5
Cefepime		149	59.1	20.8	20.1	8	32	<0.5	>32
Ceftazidime		149	59.7	10.7	29.5	<8	>32	<8	>32
Ceftriaxone		149	18.1	26.8	55	64	>64	0.5	>64
Imipenem		148	75	14.9	10.1	1	16	0.5	>16
Levofloxacin		149	51	4.7	44.3	2	>8	0.06	>8
Minocycline		149	3.4	16.8	79.9	>16	>16	1	>16
PipTazo		149	85.9	0	14.1	8	128	<0.06	>128
Tigecycline		149	na	na	na	8	>16	0.12	>16

Table 3. In vitro activity of tigecycline and comparator agents against gram positive pathogens

Organism	Drug	N=	%Sus	%Int	%Res	MIC ₅₀	MIC ₉₀	LOW	HIGH
<i>Staphylococcus aureus</i> , MRSA	AmoxClav	95	13.7	0	86.3	>8	>8	1	>8
	Ampicillin	95	0	0	100	>16	>16	2	>16
	Ceftriaxone	95	6.3	9.5	84.2	>64	>64	4	>64
	Imipenem	95	23.2	4.2	72.6	>16	>16	<0.12	>16
	Levofloxacin	95	14.7	11.6	73.7	4	16	0.12	32
	Linezolid	95	100	0	0	2	2	<0.5	4
	Minocycline	95	97.9	2.1	0	<0.25	4	<0.25	8
	Penicillin	95	0	0	100	>8	>8	4	>8
	PipTazo	95	13.7	0	86.3	>16	>16	1	>16
	Tigecycline	95	98.9	0	1.1	0.12	0.25	0.06	1
<i>Staphylococcus aureus</i> , MSSA	AmoxClav	108	100	0	0	1	1	0.12	4
	Ampicillin	108	13.9	0	86.1	2	>16	<0.06	>16
	Ceftriaxone	108	100	0	0	4	4	1	