

REVISED ABSTRACT

Background: Tigecycline, a glycylcycline, has demonstrated potent activity against a range of pathogens encountered in hospitalized patients. The TEST program was designed to determine the in vitro activity of tigecycline and comparators to nosocomial pathogens isolated in Italy between 2004-2006. **Methods:** Over 965 clinical isolates were collected and identified at each site and confirmed by the reference lab. Following CLSI guidelines, MICs were determined and interpreted using supplied broth microdilution panels. **Results:** The spectrum and potency of tigecycline against nosocomial pathogens is shown in the table below:

Organism (#)	Tigecycline %S	MIC ₉₀	% Inhibited at MIC			
			≤0.25	0.5	1	2
<i>Acinetobacter</i> spp. (68)	NA	1	53	85	99	100
<i>Enterobacteriaceae</i> (420)	94	1	40	70	91	94
ESBL producers ^a (45)	100	2	53	78	87	100
<i>Enterobacter</i> spp. (122)	96	2	60	86	93	99
<i>E. faecalis</i> (41)	95	0.25	100	-	-	-
<i>E. faecium</i> (29)	100	0.25	100	-	-	-
<i>H. influenzae</i> (72)	NA	0.25	90	97	100	-
<i>P. aeruginosa</i> (100)	NA	>16	-	-	4	14
<i>S. aureus</i> (MSSA) (89)	100	0.25	30	83	99	100
<i>S. aureus</i> (MRSA) (54)	96	0.5	-	7	56	93
<i>S. pneumoniae</i> (70)	NA	1	40	71	100	-
<i>S. agalactiae</i> (41)	100	0.06	100	-	-	-

^aESBL producing *E. coli*, *K. oxytoca*, *K. pneumoniae*

Conclusion: Italian isolates of gram-positive and -negative hospital pathogens demonstrated excellent tigecycline MIC₉₀s, excluding *P. aeruginosa*. For most resistant phenotypes TIG MIC₉₀s were 1 mcg/ml or less, and the majority of isolates were inhibited at 2 mcg/ml or less. All ESBLs isolated in Italy were inhibited by tigecycline at MICs of 2 or less. Tigecycline promises expanded broad spectrum coverage against multiply resistant pathogens isolated in Italy.

INTRODUCTION

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 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].

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 Italian laboratories. This study is part of the larger ongoing global Tigecycline Evaluation and Surveillance Trials (T.E.S.T.) program.

RESULTS

The results are listed in the following tables.

Table 1. In vitro activity of tigecycline and comparative agents against 495 strains of *Enterobacteriaceae*.

Organism Name ^a	Drug	%SUS ^b	%INT	%RES	MIC (mcg/ml)	
					MIC ₅₀	MIC ₉₀
All <i>Enterobacteriaceae</i> (n=420)	Tigecycline	94	5	1	0.5	1
	Amikacin	96.7	2.6	0.7	2	16
	AmoxClav	35.2	12.9	51.9	32	>32
	Ampicillin	10.2	5	84.8	>32	>32
	Cefepime	87.1	4	8.8	<0.5	16
	Ceftazidime	62.9	4	33.1	<0.5	>32
	Ceftriaxone	69.8	8.8	21.4	0.5	>64
	Imipenem	100	0	0	0.5	1
	Levofloxacin	72.1	3.6	24.3	0.12	>8
	Minocycline	71.7	13.6	14.8	4	16
	PipTazo	73.3	8.6	18.1	4	>128
<i>E. coli</i> (n=127)	Tigecycline	98.4	0.8	0.8	0.25	0.5
	Amikacin	100	0	0	2	8
	AmoxClav	53.5	18.9	27.6	8	32
	Ampicillin	33.1	0	66.9	>32	>32
	Cefepime	90.6	1.6	7.9	<0.5	8
	Ceftazidime	82.7	3.9	13.4	<0.5	>32
	Ceftriaxone	82.7	3.1	14.2	<0.06	64
	Imipenem	100	0	0	0.25	0.5
	Levofloxacin	65.4	2.4	32.3	0.06	>8
	Minocycline	69.3	10.2	20.5	2	>16
	PipTazo	85.8	3.9	10.2	2	>128
<i>K. pneumoniae</i> (n=90)	Tigecycline	93.3	5.8	1.1	0.5	2
	Amikacin	93.3	6.7	0	2	16
	AmoxClav	60	20	20	8	32
	Ampicillin	0	11.1	88.9	>32	>32
	Cefepime	83.3	8.9	7.8	<0.5	16
	Ceftazidime	60	2.2	37.8	<0.5	>32
	Ceftriaxone	72.2	7.8	20	0.12	64
	Imipenem	100	0	0	0.5	1
	Levofloxacin	76.7	8.9	14.4	0.06	>8
	Minocycline	71.1	7.8	21.1	4	16
	PipTazo	76.7	8.9	14.4	2	>128
<i>K. oxytoca</i> (n=30)	Tigecycline	83.3	6.7	0	0.5	2
	Amikacin	100	0	0	2	8
	AmoxClav	53.3	23.3	23.3	4	32
	Ampicillin	0	6.7	93.3	>32	>32
	Cefepime	83.3	0	6.7	<0.5	4
	Ceftazidime	83.3	3.3	13.3	<0.5	>32
	Ceftriaxone	83.3	3.3	13.3	0.25	64
	Imipenem	100	0	0	0.5	0.5
	Levofloxacin	86.7	0	13.3	0.06	8
	Minocycline	86.7	6.7	6.7	2	8
	PipTazo	60	3.3	36.7	4	>128
ESBL-producing <i>E. coli</i> , <i>K. pneumoniae</i> , <i>K. oxytoca</i> (n=45)	Tigecycline	100	0	0	0.25	2
	Amikacin	88.9	11.1	0	8	32
	AmoxClav	31.1	33.3	35.6	16	>32
	Ampicillin	0	0	100	>32	>32
	Cefepime	64.4	13.3	22.2	8	>32
	Ceftazidime	11.1	6.7	82.2	>32	>32
	Ceftriaxone	31.1	20	48.9	32	>64
	Imipenem	100	0	0	0.25	0.5
	Levofloxacin	42.2	8.9	48.9	4	>8
	Minocycline	60	8.9	31.1	4	>16
	PipTazo	51.1	17.8	31.1	16	>128
<i>E. aerogenes</i> (n=47)	Tigecycline	97.9	2.1	0	0.5	2
	Amikacin	91.5	8.5	0	8	16
	AmoxClav	10.6	6.4	83	>32	>32
	Ampicillin	0	2.1	97.9	>32	>32
	Cefepime	83	2.1	14.9	2	>32
	Ceftazidime	25.5	4.3	70.2	>32	>32
	Ceftriaxone	46.8	27.7	25.5	16	>64
	Imipenem	100	0	0	1	2
	Levofloxacin	53.2	4.3	42.6	0.25	>8
	Minocycline	76.6	21.3	2.1	4	8
	PipTazo	70.2	14.9	14.9	8	>128
<i>E. cloacae</i> (n=74)	Tigecycline	66.5	10.8	2.7	1	4
	Amikacin	95.9	1.4	2.7	2	8
	AmoxClav	2.7	0	97.3	>32	>32
	Ampicillin	0	5.4	94.6	>32	>32
	Cefepime	82.4	6.8	10.8	2	32
	Ceftazidime	39.2	6.8	54.1	32	>32
	Ceftriaxone	44.6	10.8	44.6	32	>64
	Imipenem	100	0	0	0.5	2
	Levofloxacin	70.3	1.4	28.4	0.12	>8
	Minocycline	68.9	16.2	14.9	4	16
	PipTazo	43.2	18.9	37.8	32	>128
<i>S. marcescens</i> (n=47)	Tigecycline	93.6	6.4	0	1	2
	Amikacin	100	0	0	4	8
	AmoxClav	0	4.3	95.7	>32	>32
	Ampicillin	0	2.1	97.9	>32	>32
	Cefepime	93.6	0	6.4	<0.5	8
	Ceftazidime	74.5	4.3	21.3	<0.5	>32
	Ceftriaxone	83	8.5	8.5	0.5	32
	Imipenem	100	0	0	1	2
	Levofloxacin	93.6	2.1	4.3	0.12	1
	Minocycline	68.1	27.7	4.3	4	8
	PipTazo	91.5	2.1	6.4	2	16

^aInterpretive criteria as defined by CLSI M100-S16 (2006), where available; tigecycline susceptibility breakpoints are according to FDA package insert (Tysabri[®], 2005), where available [9].
^bSpecies with n < 10 were omitted.

Table 2. In vitro activity of tigecycline and comparative agents against *Acinetobacter* spp. and *P. aeruginosa*.

Organism Name	Drug	%SUS ^b	%INT	%RES	MIC (mcg/ml)		
					MIC ₅₀	MIC ₉₀	
<i>Acinetobacter</i> spp. (n=68)	Tigecycline	na	na	na	0.25	1	
	Amikacin	66.2	8.8	25	4	>64	
	Cefepime	47.1	17.6	35.3	16	>32	
	Ceftazidime	45.6	4.4	50	16	>32	
	Ceftriaxone	35.3	19.1	45.6	32	>64	
	Imipenem	80.9	10.3	8.8	0.5	8	
	Levofloxacin	58.8	5.9	35.3	2	>8	
	Minocycline	100	0	0	<0.5	2	
	PipTazo	66.2	4.4	29.4	8	>128	
	<i>P. aeruginosa</i> (n=120)	Tigecycline	na	na	na	16	>16
		Amikacin	88	6	6	4	32
Cefepime		52	22	26	8	>32	
Ceftazidime		65	10	25	<0.5	>32	
Ceftriaxone		9	17	74	>64	>64	
Imipenem		72.2	9.3	18.6	2	>16	
Levofloxacin		52	5	43	2	>8	
Minocycline		2	10	88	>16	>16	
PipTazo		74	0	26	16	>128	

^aInterpretive criteria as defined by CLSI M100-S16 (2006), where available; tigecycline susceptibility breakpoints are according to FDA package insert (2005), where available [9]; na = not available; breakpoints are not yet established against this species.

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 from 2004 to 2006 from 5 study centers across Italy. 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); ceftriaxone (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 ceftriaxone 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

- Hoffman, 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. 1085-9.
- Lattavakul, 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. 3867-9.
- Projan, S.J. Preclinical pharmacology of GAR-936, a novel glycylcycline antibacterial agent. *Pharmacotherapy.* 2000, 20(9 Pt 2): p. 2198-223S; discussion 224S-228S.
- Chen, 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.
- 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.
- Rupp, M.E. and P.D. Ten. 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. 2006. Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
- Tysabri. 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.

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

Organism Name	Drug	%SUS ^b	%INT	%RES	MIC (mcg/ml)	
					MIC ₅₀	MIC ₉₀
<i>S. aureus</i> (MRSA) (n=54)	Tigecycline	96.3	0	3.7	0.25	0.5
	AmoxClav	0	0	100	>8	>8
	Ampicillin	0	0	100	>16	>