

REVISED ABSTRACT

Background: Efficacy of antimicrobials continues to be eroded by development and spread of bacterial resistance. Surveillance studies can help guide appropriate use of antimicrobials by understanding current trends in susceptibility. The Tigecycline Evaluation Surveillance Trial (TEST) is an ongoing global study that can serve to help recognize these trends on many levels. This report evaluates differences in susceptibility of strains collected from different body sites by investigators in South America 2004-2006. **Methods:** 1,267 strains isolated from 10 specimen types were collected and identified from 2004-2006 at 10 hospitals in Argentina and Brazil. MICs for each strain were determined per CLSI guidelines at each facility using broth microdilution. MIC_{50/90} values were analyzed to identify any significant differences in antibiograms from different sources. **Results:** Minimal differences were seen in susceptibility patterns of evaluated pathogens and specimen sources. MIC_{50/90} values for all organism/specimen source pairings were usually +/- 2 dilutions of each other, with no single source giving higher MIC_{50/90} values than others. TIG MIC₉₀/MIC₅₀ ratios were usually much lower than those of the comparators, especially for species frequently resistant to routinely-used antimicrobials. **Conclusion:**Antibiograms of bacteria isolated from 10 different body sites were generally similar, with no single source showing significantly different sensitivity patterns. TIG's demonstrated broad spectrum of activity and consistently low MIC₉₀/MIC₅₀ ratios, including strains resistant to other drugs (MRSA, ESBL-producers, *Acinetobacter*, *E. faecium*, etc.), should establish its role as an important addition to hospital formularies.

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 considered a bacteriostatic agent, it has shown some bactericidal activity against key 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 of less than 10⁻⁹ observed [3, 5, 7]. Tetracycline-resistant bacteria with either tetracycline efflux pumps or ribosomal protective features are usually sensitive to tigecycline [2-4, 7-11], except for *Pseudomonas aeruginosa*. Tigecycline has been shown to be highly effective against multi-resistant *Acinetobacter* spp., particularly *A. baumannii* that are commonly associated with serious nosocomial infections. Similar activity has been observed against *Enterobacteriaceae*, including most extended-spectrum beta-lactamase (ESBL) producing strains [10]. Tigecycline has demonstrated MIC₉₀ values of <=0.5 mcg/ml against methicillin-resistant *Staphylococcus 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 *Enterococcus faecalis* [4, 5] with diverse genotypes van-A, -B and -C [6].

With such a broad spectrum of activity, tigecycline has the potential to be useful in a variety of infections. This report describes the in vitro activity of tigecycline against a large, diverse population of clinical isolates collected from various specimen types in hospitals in South America from 2004 through 2006.

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.
- 1,267 clinical isolates were collected and tested between January 2004 and June 2006 from 10 study centers in Argentina and Brazil
- 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 breakpoints (in units of mcg/ml), as approved by the US Food and Drug Administration (FDA), are defined as follows: *Enterobacteriaceae*: susceptible <=2, intermediate =4, and resistant >=8; *Staphylococcus aureus* (including MRSA): susceptible <=0.5, no intermediate or resistant breakpoints; *Enterococcus faecalis* (vancomycin-susceptible): susceptible <=0.25, no intermediate or resistant breakpoints; non-pneumococcal streptococci: susceptible <=0.25, no intermediate or resistant breakpoints.
- 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; *Haemophilus 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.
- Albanan, D., M. Macheling, and K. Bush, Novel antibacterial agents for the treatment of serious Gram-positive infections. *Expert Opin Investig Drugs*, 2003, 12(3): p. 379-99.
- Bertru, 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.
- Gates, 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. 2195-223S; 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-butylglycylamide 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. 2656-60.
- Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing: Sixteenth International Supplement. CLSI document M100-S16. Wayne, PA, 2006.

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RESULTS

Table 1. Tigecycline MIC₅₀ and MIC₉₀ (mcg/ml) for gram-negative isolates with n<=10 from various body sites^a. (GI = Gastrointestinal; GU = Genitourinary; HEENT = Head, Ears, Eyes, Nose, Throat; Resp = Respiratory; SSS = Skin/Soft Tissue)

Organism		Blood	Cath/Drain	GI	GU	Resp	SSS
<i>E. coli</i>	(n)	(49)		(44)	(32)	(12)	(22)
	MIC ₅₀	0.12		0.12	0.12	0.25	0.12
	MIC ₉₀	0.25		0.5	0.25	0.25	0.25
<i>Klebsiella</i> spp.	(n)	(46)	(11)	(23)	(26)	(40)	(16)
	MIC ₅₀	0.5	1	0.5	0.5	0.5	0.5
	MIC ₉₀	1	2	1	4	1	2
ESBL-producers	(n)	(19)		(13)	(10)	(17)	
	MIC ₅₀	0.5		0.5	0.5	1	
	MIC ₉₀	2		1	4	2	
<i>Enterobacter</i> spp.	(n)	(32)		(28)	(16)	(18)	(38)
	MIC ₅₀	0.1		0.5	1	0.5	0.5
	MIC ₉₀	1		1	4	1	2
<i>Serratia</i> spp.	(n)	(13)					(13)
	MIC ₅₀	1					0.5
	MIC ₉₀	2					1
<i>P. aeruginosa</i>	(n)	(34)	(10)			(54)	(25)
	MIC ₅₀	8	16			8	8
	MIC ₉₀	>16	>16			16	16
<i>Acinetobacter</i> spp.	(n)	(13)	(16)			(30)	(18)
	MIC ₅₀	0.5	1			0.5	0.5
	MIC ₉₀	1	2			2	1
<i>H. influenzae</i>	(n)	(10)				(72)	
	MIC ₅₀	0.25				0.25	
	MIC ₉₀	0.5				0.5	
<i>H. influenzae</i> (beta-lac positive)	(n)					(10)	
	MIC ₅₀					0.5	
	MIC ₉₀					0.5	

^aMIC₉₀ not calculated if n<=10.

Table 2. Tigecycline MIC₅₀ and MIC₉₀ (mcg/ml) for gram-positive isolates with n<=10 from various body sites^a.

Organism		Blood	Bone	Cath/Drain	GU	Resp	SSS
<i>S. aureus</i>	(n)	(76)	(10)	(20)	(39)	(47)	
	MIC ₅₀	0.12	0.12	0.12		0.12	0.12
	MIC ₉₀	0.25	0.25	0.25		0.25	0.5
<i>S. aureus</i> MRSA	(n)	(26)		(13)	(20)	(23)	
	MIC ₅₀	0.12		0.12	0.12	0.12	
	MIC ₉₀	0.25		0.25	0.25	0.25	
<i>E. faecalis</i>	(n)	(11)			(16)		(13)
	MIC ₅₀	0.12			0.12		0.06
	MIC ₉₀	0.12			0.25		0.12
All VRE ^b	(n)						
	MIC ₅₀						
	MIC ₉₀						
<i>S. pneumoniae</i>	(n)	(28)				(33)	
	MIC ₅₀	0.5				0.25	
	MIC ₉₀	1				1	
<i>S. pneumoniae</i> ^c (pen-intermed.)	(n)						
	MIC ₅₀						
	MIC ₉₀						
<i>S. pneumoniae</i> ^d (pen-resistant)	(n)						
	MIC ₅₀						
	MIC ₉₀						
<i>S. agalactiae</i>	(n)				(36)		
	MIC ₅₀				0.03		
	MIC ₉₀				0.03		

^aMIC₉₀ not calculated if n<=10.

^bAll n's were <=10; MICmin/max for all 7 VRE from all body sites was 0.03-0.25.

^cAll n's were <=10; MICmin/max for all 16 strains from all body sites was 0.015-1.

^dAll n's were <=10; MICmin/max for all 7 strains from all body sites was 0.12-1.

Tables 3a - 3k. Drugs with MIC₅₀ or MIC₉₀ varying by >2 log₂ dilutions among specimen sources (n<=10). Ak=amikacin, Am=ampicillin, AUG=amoxicillin/clavulanic acid, Cpe=cefepime, Cax=ceftriaxone, Caz=ceftazidime, Imp=imipenem, Lvx=levofloxacin, Min=minocycline, P=penicillin, P/T=piperacillin/tazobactam, Va=vancomycin.

Table 3a. *E. coli* MIC_{50/90}

Source (n)	Cpe	Cax	P/T
Blood (49)	<=0.5/2	<=0.06/8	1/4
GI (44)	<=0.5/<=0.5	<=0.06/0.25	1/16
GU (42)	<=0.5/<=0.5	<=0.06/0.12	1/2
Resp (12)	<=0.5/32	<=0.06/>64	1/16
SSS (22)	<=0.5/<=0.5	<=0.06/0.12	1/2

Table 3b. *Klebsiella* spp. MIC_{50/90}

Source (n)	AUG	Cpe	Cax	Lvx	P/T
Blood (46)	4/32	<=0.5/>32	<=0.06/>64	0.06/4	2/128
Cath/Drain (11)	32/32	4/>32	32/>64	1/>8	8/>128
GI (23)	4/32	8/>32	32/>64	0.12/8	2/128
GU (26)	16/>32	<=0.5/>32	0.12/>64	0.06/>8	4/>128
Resp (40)	32/>32	2/>32	0.25/>64	0.06/>8	2/>128
SSS (16)	4/>32	<=0.5/>32	<=0.06/>64	0.06/>8	1/128

Table 3c. *Enterobacter* spp. MIC_{50/90}

Source (n)	Ak	Cpe	Cax	Lvx	Min	P/T
Blood (32)	2/16	<=0.5/16	0.25/64	0.03/>8	2/8	2/64
GI (28)	2/32	<=0.5/>32	1/>64	0.06/4	4/16	2/128
GU (16)	2/16	1/>32	4/>64	0.12/>8	4/>16	8/128
Resp (18)	2/4	<=0.5/8	0.12/32	0.03/0.25	2/4	1/64
SSS (38)	2/64	<=0.5/>32	0.5/>64	0.06/8	4/>16	2/128

Table 3d. *Serratia* spp. MIC_{50/90}

Source (n)	Cpe	Cax	Lvx	P/T
Blood (13)	<=0.5/16	1/>64	0.12/2	2/64
SSS (13)	<=0.5/1	0.12/1	0.06/0.12	1/2

Table 3e. *Acinetobacter* spp. MIC_{50/90}

Source (n)	Imp
Blood (13)	2/>16
Cath/Drain (16)	16/>16
Resp (30)	16/>16
SSS (18)	1/>16

Table 3f. *Pseudomonas aeruginosa* MIC_{50/90}

Source (n)	Lvx	P/T
Blood (34)	8/>8	4/128
Cath/Drain (10)	1/>8	32/128
Resp (54)	2/>8	8/128
SSS (25)	2/>8	8/64

Table 3g. *Enterococcus faecalis* MIC_{50/90}

Source (n)	Lvx
Blood (11)	1/32
GU (16)	1/1
SSS (13)	0.5/1

Table 3h. *Streptococcus pneumoniae* MIC_{50/90}

Source (n)	Min
Blood (28)	<=0.25/<=0.25
Resp (33)	<=0.25/4

Table 3i. *Staphylococcus aureus* MIC_{50/90}

Source (n)	AUG	Cax	Imp	Lvx	Min	P/T
Blood (76)	1/>8	4/>64	<=0.12/>16	0.25/8	<=0.25/0.5	1/>16
Bone (10)	>8/>8	>64/>64	8/>16	2/16	<=0.25/4	>16/>16
Cath/Drain (20)	4/>8	8/>64	0.25/>16	0.5/8	<=0.25/4	2/>16
Resp (39)	4/>8	16/>64	0.25/>16	1/8	<=0.25/4	8/>16
SSS (47)	2/>8	4/>64	0.25/>16	0.25/4	<=0.25/1	2/>16

Table 3k. *Haemophilus influenzae* MIC_{50/90}

Source (n)	Am
Blood (10)	<=0.5/1
Resp (72)	<=0.5/8

CONCLUSIONS

- Tigecycline MIC₅₀ values for each species/group were within 2 log₂ dilutions, demonstrating remarkable consistency regardless of specimen source.
- Tigecycline MIC₉₀ values for strains of each species/group isolated from various sources were also within 2 log₂ dilutions, except for ESBL-producers and *Enterobacter* spp. from the genitourinary tract, where the MIC₉₀ ranges were 4 doubling dilutions.
- Tigecycline MIC₉₀ values were within 1-2 log₂ dilutions of the MIC₅₀ in almost all cases, with the exception of ESBL-producing *K. pneumoniae* isolated from the genitourinary tract, where up to 3 doubling dilutions separated them.

- Most MIC₅₀ values for comparator drugs were within 2 doubling dilutions across specimen sources; however, there were numerous observations of MIC₉₀s varying by more than 2 log₂ dilutions across specimen sources. Nevertheless, with the exception of *Serratia* spp. blood isolates of which were more resistant than those from skin and skin structures, there was rarely a clear trend for any given source to consistently yield more