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In Vitro Activity of Tigecycline Tested Against an International Collection of Minocycline-Resistant Bacterial Pathogens (2000-2004)

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AMENDED ABSTRACT

Objective:

To characterize the activity of tigecycline (TIG) against an international collection of minocycline (MINO)-susceptible (S) and -resistant (R) Gram-positive and -negative pathogens. TIG, a novel glycylcycline, is the 9-butylglycylamido derivative of MINO and has recently been approved by the US-FDA as a parenteral agent for use in treatment of skin and skin structure infections, and intra-abdominal sepsis. Glycylcyclines are inherently stable to most tetracycline-class resistance mechanisms.

Methods:

Bacterial strains were acquired from recent (2000 to 2004) worldwide surveillance collections, and tested against TIG, MINO and other comparator agents using the NCCLS [2003] reference broth microdilution method. Interpretive criteria were from the TIG package insert or CLSI [2005].

Results:

Activity of TIG is summarized in the table.

Organism (no. MINO-S/MINO-R)	% TIG-R	TIG $MIC_{50/90}$ in mg/L (%S)	
		MINO-S isolates	MINO-R isolates
E. coli (EC; 1,821/271)	0.0	0.12/0.25 (100)	0.25/0.5 (100)
Klebsiella spp. (861/147)	0.0	0.5/0.5 (100)	0.5/4 (86.4)
Enterobacter spp. (ESP; 572/66)	0.0	0.5/1 (99.8)	2/4 (63.6)
P. mirabilis (PM; 7/221)	9.0	0.5/1 (100)	4/8 (43.4)
Proteus, indole + (IPP; 54/56)	4.9	1/1 (98.1)	2/8 (64.3)
Serratia marcesens (SM; 209/24)	3.3	1/1 (99.0)	4/8 (29.2)
P. aeruginosa spp. (189/676)	-[a]	2/4 (-n)	16/32 (-n)
Acinetobacter spp. (409/8)	-[a]	1/2 (-n)	1/4 (-n)
S. aureus (SA; 5,243/34)	-[b]	0.12/0.25 (99.9)	0.25/0.5 (94.1)
E. faecalis (EF; 302/475)	-[b]	0.06/0.25 (97.0)	0.12/0.25 (93.9)
E. faecium (EFM; 264/97)	-[b]	0.06/0.25 (99.6)	0.06/0.25 (99.0)

a. Breakpoint criteria have not been established
b. Resistant breakpoint criteria have not been established

TIG was highly active against all strains tested with defined resistance only being seen among the Proteae and SM (3.3 to 9.0%). Despite close structural similarities between the two molecules, TIG remained generally active against most MINO-S and -R organisms with MIC_{50} values being 0.06 to 2 mg/L, and 0.06 to 4 mg/L (exception, PSA, MIC_{50}, 16 mg/L), respectively. Among MINO-R subsets, >93% of such critical pathogens as EC, SA, EF and EFM remained S to TIG. Decreases in TIG susceptibilities were noted among MINO-R SM (29.2%), PM (43.3%), IPP (64.3%) and ESP (63.6%), indicative of over-lapping binding sites.

Conclusions:

TIG offers advantages to existing agents including an enhanced spectrum of activity and stability to the commonly occurring tetracycline class R mechanisms (*tet*A or B and *tet*M or O). Although a derivative of MINO, TIG retains activity against many MINO-R isolates, making this first-in-class glycylcycline an attractive candidate for expanded clinical development.

INTRODUCTION

Members of the tetracycline class are broad-spectrum antimicrobics and have been used successfully since the late 1940's to treat a variety of community- and hospital-acquired infections. Resistance occurs commonly however, and has increasingly limited the usefulness of the class in human medicine. The development of newer agents with similar spectrum of activity and safety profiles to the tetracyclines but with stability to the commonly occurring resistance mechanisms has become a priority, and has led to the discovery of synthetic analogues known as the glycylcyclines.

Tigecycline (formerly GAR-936), the 9-t-butylglycylamido derivative of minocycline, is the first member of the glycylcycline class to be approved by the United States-Food and Drug Administration (US-FDA) as a parenteral agent for the treatment of complicated skin and skin structure infections, and intra-abdominal infections. Tigecycline offers important advantages to existing antimicrobials including enhanced spectrum of activity and stability against the highly prevalent tetracycline resistance mechanisms (Tet A or B efflux determinants and Tet M or O ribosomal protection factors).

In this study, we evaluated the in vitro activity of tigecycline tested against a collection of 13,385 Gram-positive and -negative isolates (including 2,075 minocycline-resistant strains) recovered predominantly from patients with bloodstream, respiratory tract, skin and soft tissue and urinary tract infections, to specifically determine the influence that tetracycline (minocycline) resistance has on tigecycline susceptibility in contemporary clinical isolates.

MATERIALS AND METHODS

Bacterial Strains: To assess the spectrum of activity and potency of tigecycline, recent clinical isolates submitted to a reference laboratory (JMI Laboratories, North Liberty, Iowa, USA) were examined. A total of 13,385 isolates (including 2,075 minocycline-resistant strains) of Enterobacteriaceae (4,716), non-fermentative bacilli (1,868), staphylococci (5,388) and enterococci (1,413) were studied. All patient isolates were consecutively acquired, non-duplicate and were submitted from > 70 participating medical centers representing 29 countries in Asia - Australia, Europe, South America and North America. Isolates were identified by the submitting laboratory and confirmed by the monitoring facility (JMI Laboratories) using standard biochemical algorithms. The collection consisted of those species and resistant subsets as specified in Tables 1 and 2.

Susceptibility Testing: MIC values were determined for tigecycline, minocycline and comparator agents using "validated", dry-form broth microdilution panels with cation-adjusted Mueller-Hinton medium. Testing, incubation and MIC interpretations were performed using the recommendations of the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS). Quality control strains utilized included *Escherichia coli* ATCC 25922 and 35218, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853; all results were within CLSI specified ranges.

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RESULTS

- Among a large collection of *S. aureus* (5,388 isolates; 39.5% oxacillin-resistant) and coagulase-negative staphylococci (1,306; 79.6% oxacillin-resistant), all strains were inhibited by ≤ 1 mg/L of tigecycline (>99% susceptible); tigecycline and minocycline were the most potent agents tested (MIC_{50} and MIC_{90} values, ≤ 0.25 and ≤ 0.5 mg/L, respectively; Table 1).

- Tigecycline, vancomycin and linezolid provided the broadest coverage of staphylococci (≥ 99%; Table 1) and 94% of minocycline-resistant *S. aureus* remained susceptible to tigecycline (Table 2).

- Tigecycline was the most potent compound tested against *Enterococcus* spp. (MIC_{50} and MIC_{50} values, 0.06 and 0.25 mg/L, respectively). Despite the presence of 44.1% vancomycin resistance among *E. faecium*, ≥ 99.3% of strains remained susceptible to tigecycline; only linezolid performed similarly (Table 1).

- Susceptibility of enterococci to tigecycline was indifferent to the presence of minocycline resistance (Table 2).

- *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. were largely susceptible to tigecycline (100.0%, 98.2% and 96.3%, respectively); while tigecycline potency among minocycline-resistant isolates decreased by two- to eight-fold, no MIC value above 4 mg/L was observed (0.0% resistance).

- Among Enterobacteriaceae, only imipenem was consistently more potent than comparators and displayed greater coverage (≥ 98% susceptibility). Only *P. mirabilis* and other Proteae displayed consistently elevated tigecycline MIC values (MIC_{90}, 4 mg/L).

- Among non-fermentative bacilli, most *Acinetobacter* spp. were inhibited at achievable serum concentrations of tigecycline (MIC_{50} and MIC_{90}, 1 and 2 mg/L, respectively); resistance to minocycline, while rare (1.8%), appeared to have little impact on the potency of tigecycline (MIC_{50}, 1 mg/L).

- *P. aeruginosa* were recognized to be minimally inhibited by tigecycline (MIC_{90}, 16 mg/L); a further decrease in tigecycline activity (MIC_{90}, 32 mg/L) was largely associated with the minocycline-resistant (47.2% of isolates) *P. aeruginosa* strains.

Table 1. Antimicrobial activity of tigecycline, minocycline and selected comparators tested against the ranking Gram-positive and -negative bacterial pathogens collected as part of international surveillance studies (2000 - 2004).

Organism (no. tested)/ Antimicrobial agent	MIC (mg/L)			% by category[a]	
	50%	90%	Range	Susceptible	Resistant
S. aureus (5,388)					
Tigecycline	0.12	0.25	≤0.016-1	99.9	-[b]
Minocycline	≤0.25	≤0.25	≤0.25->8	97.3	0.6
Tetracycline	≤0.25	>8	≤0.25->8	89.4	10.2
Oxacillin	0.5	>2	≤0.25->2	60.5	39.5
Erythromycin	0.5	>8	≤0.06->8	52.7	46.3
Clindamycin	0.12	>8	≤0.06->8	74.7	25.0
Ciprofloxacin	0.5	>4	≤0.03->4	63.4	35.7
Linezolid	2	2	0.12-4	100.0	-
Vancomycin	1	1	0.25-4	100.0	0.0
Coagulase-negative staphylococci (1,306)					
Tigecycline	0.12	0.5	≤0.016-1	99.5	-
Minocycline	≤0.25	0.5	≤0.25->8	99.7	0.2
Tetracycline	1	>8	≤0.25->8	82.8	16.4
Oxacillin	>2	>2	≤0.25->2	20.4	79.6
Erythromycin	>8	>8	≤0.06->8	33.0	66.5
Clindamycin	≤0.06	>8	≤0.06->8	63.8	35.3
Ciprofloxacin	2	>4	0.06->4	49.0	47.2
Linezolid	1	1	0.25-2	100.0	-
Vancomycin	1	2	≤0.12-4	100.0	0.0
E. faecalis (1,000)					
Tigecycline	0.06	0.25	≤0.016-0.5	95.6	-
Minocycline	>8	>8	≤0.25->8	30.2	47.5
Tetracycline	>8	>8	≤0.25->8	26.9	72.9
Ampicillin	≤1	2	≤1->16	99.4	0.6
Quin/Dalfo	>2	>2	0.5->2	0.4	96.2
Linezolid	2	2	0.5->8	99.6	0.4
Vancomycin	1	2	0.25->16	97.7	2.2
E. faecium (413)					
Tigecycline	0.06	0.25	≤0.016-0.5	99.3	-
Minocycline	≤0.25	>8	≤0.25->8	63.9	23.5
Tetracycline	0.5	>8	≤0.25->8	59.8	39.7
Ampicillin	>16	>16	≤1->16	10.7	89.3
Quin/Dalfo	0.5	2	≤0.25->2	84.3	6.1
Linezolid	2	2	0.5-8	99.8	0.0
Vancomycin	1	>16	0.25->16	55.4	44.1

Organism (no. tested)/ Antimicrobial agent	MIC (mg/L)			% by category[a]	
	50%	90%	Range	Susceptible	Resistant
E. coli (2,285)					
Tigecycline	0.12	0.25	0.03-2	100.0	0.0
Minocycline	1	>8	≤0.25->8	79.7	11.9
Tetracycline	2	>8	≤0.25->8	64.2	35.1
Ceftazidime	≤1	≤1	≤1->16	95.3	3.2
Cefepime	≤0.12	0.25	≤0.12->16	96.6	2.8
Pip/Tazo	2	8	≤0.5->64	94.3	2.6
Imipenem	≤0.12	0.25	≤0.12-2	100.0	0.0
Ciprofloxacin	≤0.03	>4	≤0.03->4	82.5	17.5
Gentamicin	≤2	4	≤2->8	91.1	8.1
Klebsiella spp. (1,084)					
Tigecycline	0.5	1	0.06-4	98.2	0.0
Minocycline	2	>8	≤0.25->8	79.4	13.6
Tetracycline	2	>8	≤0.25->8	78.7	18.0
Ceftazidime	≤1	>16	≤1->16	83.4	14.0
Cefepime	≤0.12	16	≤0.12->16	88.7	9.7
Pip/Tazo	4	>64	≤0.5->64	83.2	12.7
Imipenem	≤0.12	0.25	≤0.12->8	99.3	0.3
Ciprofloxacin	≤0.03	4	≤0.03->4	87.7	10.2
Gentamicin	≤2	>8	≤2->8	81.9	16.3
Enterobacter spp. (669)					
Tigecycline	0.5	1	0.06-4	96.3	0.0
Minocycline	2	8	≤0.25->8	85.5	9.9
Tetracycline	2	>8	0.5->8	85.4	11.2
Ceftazidime	≤1	>16	≤1->16	72.8	22.3
Cefepime	≤0.12	2	≤0.12->16	95.5	3.3
Pip/Tazo	4	>64	≤0.5->64	75.8	11.8
Imipenem	0.25	1	≤0.12->8	99.3	0.1
Ciprofloxacin	≤0.03	2	≤0.03->4	88.8	9.4
Gentamicin	≤2	8	≤2->8	88.6	9.7
P. mirabilis (279)					
Tigecycline	2	4	0.5-16	53.0	9.0
Minocycline	>8	>8	4->8	2.5	79.2
Tetracycline	>8	>8	2->8	1.4	98.2
Ceftazidime	≤1	≤1	≤1->16	98.6	1.1
Cefepime	≤0.12	0.25	≤0.12->16	96.1	3.9
Pip/Tazo	≤0.5	1	≤0.5-16	100.0	0.0
Imipenem	1	2	≤0.12-4	100.0	0.0
Ciprofloxacin	≤0.03	4	≤0.03->4	79.6	15.4
Gentamicin	≤2	8	≤2->8	89.2	10.0

Organism (no. tested)/ Antimicrobial agent	MIC (mg/L)			% by category[a]	
	50%	90%	Range	Susceptible	Resistant
Indole-positive *Proteae* (123)					
Tigecycline	1	4	0.25-16	80.5	4.9
Minocycline	8	>8	≤0.25->8	43.9	45.5
Tetracycline	>8	>8	0.5->8	38.2	53.7
Ceftazidime	≤1	4	≤1->16	97.6	1.6
Cefepime	≤0.12	4	≤0.12->16	91.1	7.3
Pip/Tazo	≤0.5	4	≤0.5->64	99.2	0.8
Imipenem	1	2	≤0.12->8	98.4	0.8
Ciprofloxacin	≤0.03	>4	≤-0.03->4	77.2	21.1
Gentamicin	≤2	>8	≤2->8	82.1	17.1
Serratia spp. (276)					
Tigecycline	1	2	0.5-16	92.0	3.3
Minocycline	4	8	≤0.25->8	75.7	8.7
Tetracycline	>8	>8	4->8	2.2	68.8
Ceftazidime	≤1	2	≤1-2	95.3	2.2
Cefepime	≤0.12	1	≤0.12->16	96.7	2.9
Pip/Tazo	2	32	≤0.5->64	88.4	5.1
Imipenem	0.5	1	≤0.12->8	98.6	1.4
Ciprofloxacin	0.06	1	≤0.03->4	93.5	5.4
Gentamicin	≤2	8	≤2->8	87.7	9.8
P aeruginosa (1,433)					
Tigecycline	8	16	0.12->32	-	-
Minocycline	8	>8	0.5->8	13.2	47.2
Tetracycline	>8	>8	0.5->8	3.3	77.0
Ceftazidime	4	>16	≤1->16	76.6	19.1
Cefepime	4	>16	≤0.12->16	75.9	11.8
Pip/Tazo	7	>64	≤0.5->64	82.1	17.9
Imipenem	1	>8	≤0.12->8	77.6	11.1
Ciprofloxacin	0.25	>4	≤0.03->4	69.1	26.1
Gentamicin	≤2	>8	≤2->8	75.9	20.0
Acinetobacter spp. (435)					
Tigecycline	1	2	0.06-8	-	-
Minocycline	≤0.25	4	≤0.25->8	94.0	1.8
Tetracycline	8	>8	≤0.25->8	47.0	37.6
Ceftazidime	>16	>16	≤1->16	32.9	62.3
Cefepime	>16	>16	≤0.12->16	38.2	46.0
Pip/Tazo	>64	>64	≤0.5->64	32.0	54.0
Imipenem	0.5	>8	≤0.12->8	78.2	18.9
Ciprofloxacin	>4	>4	≤0.03->4	34.7	64.6
Gentamicin	>8	>8	≤2->8	41.2	53.2

a. Categorical breakpoints those of CLSI M100-S15 [2005]; for tigecycline, breakpoints are those of the US-FDA.
b. Breakpoints have not been established by CLSI.

Table 2. Cumulative frequency distributions for minocycline-susceptible and-resistant bacterial subsets (13,385 strains; 2000 - 2004) tested against tigecycline.

Organism (no. tested)	MIC (mg/L)		Cumulative % inhibited by tigecycline at MIC (mg/L)							
	50%	90%	≤0.06	0.12	0.25	0.5	1	2	4	≥8
S. aureus										
Minocycline-susceptible (5,243)	0.12	0.25	23	76	97	>99	100			
Minocycline-resistant (34)	0.25	0.5	0	14	58	94	100			
E. faecalis										
Minocycline-susceptible (302)	0.06	0.25	65	74	97	100				
Minocycline-resistant (475)	0.12	0.25	38	68	93	100				
E. faecium										
Minocycline-susceptible (264)	0.06	0.25	65	84	>99	100				
Minocycline-resistant (97)	0.06	0.25	52	70	99	100				
E. coli										
Minocycline-susceptible (1821)	0.12	0.25	13	58	92	>99	100			
Minocycline-resistant (271)	0.25	0.5	3	42	85	96	98	100		
Klebsiella spp.										
Minocycline-susceptible (861)	0.5	0.5	<1	1	42	92	>99	100		
Minocycline-resistant (147)	0.5	4	0	<1	15	54	65	86	100	
Enterobacter spp.										
Minocycline-susceptible (572)	0.5	1	<1	1	30	89	>99	>99	100	
Minocycline-resistant (66)	2	4	0	0	3	10	25	63	100	
P. mirabilis										
Minocycline-susceptible (7)	0.5	-	0	0	0	57	100			
Minocycline-resistant (221)	4	8	0	0	0	0	1	43	89	>99
Serratia spp.										
Minocycline-susceptible (209)	1	1	0	0	0	15	93	99	100	
Minocycline-resistant (24)	4	8	0	0	0	0	4	29	62	91
P. aeruginosa										
Minocycline-susceptible (189)	2	4	0	<1	2	7	21	50	96	98
Minocycline-resistant (676)	16	32	0	<1	<1	<1	<1	1	4	39
Acinetobacter spp.										
Minocycline-susceptible (409)	1	2	4	15	31	47	68	94	>99	100
Minocycline-resistant (8)	1	-	0	0	0	0	50	87	100	

CONCLUSIONS

- Tigecycline, licensed in June of 2005 by the US-FDA, is the initial representative of the glycylcycline class and represents a novel choice among parenteral agents for broad-spectrum coverage in cases of skin and soft tissue, and intra-abdominal infections.

- Significant advantages to existing agents include an enhanced spectrum of activity and stability to the commonly occurring tetracycline class resistance mechanisms (*tet*A or B and *tet*M or O, among others).

- Although a derivative of minocycline, tigecycline retains activity against most minocycline-resistant staphylococci, enterococci, Enterobacteriaceae (except for Proteae) and *Acinetobacter* spp., making this first-in-class glycylcycline an attractive candidate for expanded clinical development.