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In Vitro Study of Tigecycline Against 776 Clinical Isolates of Non-Enterobacteriaceae From Hospitals Across Europe

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REVISED ABSTRACT

Background: Tigecycline (GAR-936) is a novel glycylcycline derivative of the tetracycline class of antimicrobials with activity against organisms with either ribosomal protection or active efflux. Tigecycline has shown activity against most members of non-Enterobacteriaceae. The activity of tigecycline was compared with those of other agents against *Acinetobacter* spp and *Pseudomonas aeruginosa* from hospitals throughout Europe. **Methods:** Clinical isolates were collected between January 2001 - September 2002. A total of 776 isolates were identified to genus and species at each site and confirmed by the central laboratory (Laboratories International for Microbiology Studies, Schaumburg, IL, USA). MIC's were determined by the central laboratory using broth microdilution panels from Dade Microscan according to NCCLS guidelines and manufacturer's instructions. **Results:** MIC_{50}s and MIC_{90}s for *Acinetobacter* spp and *Pseudomonas aeruginosa* are shown in Table 1.

Table 1. In Vitro Activity (µg/mL) of Tigecycline and Comparators Against Non-Enterobacteriaceae

	Acinetobacter spp (n=470)				*P. aeruginosa* (n=306)			
	MIC (mcg/ml)				MIC (mcg/ml)			
Antibiotic	**Range Low**	**Range High**	**MIC_{50}**	**MIC_{90}**	**Range Low**	**Range High**	**MIC_{50}**	**MIC_{90}**
Tigecycline	0.12	4	0.5	2	1	>16	>16	>16
Doxycycline	0.06	>32	0.25	>32	0.5	>32	32	>32
Amox/Clav	0.25	>64	16	>64	32	>64	>64	>64
Amp/Sulb	0.5	>32	4	>32	16	>32	>32	>32
Imipenem	0.5	>64	0.5	32	0.5	>64	1	16
Cefepime	0.5	>64	8	>64	0.5	>64	4	16
Ceftazidime	0.5	>64	8	>64	0.5	>64	2	16
Ceftriaxone	0.5	>64	16	>64	4	>64	32	>64
Levofloxacin	0.25	>64	0.5	16	0.25	>64	0.5	16

Conclusion: Tigecycline's in vitro activity against *Acinetobacter* spp is better than all other compounds tested and less than other agents tested against *P. aeruginosa*. The in vitro activity of tigecycline in this non-Enterobacteriaceae population warrants further investigation to fully understand these mechanisms of resistance.

INTRODUCTION

Glycylcyclines are tetracyclines with a 9-glycylamido substituent and were developed to avoid the two most prevalent tetracycline resistance mechanisms, ribosomal protection and efflux. Tigecycline, formerly GAR-936 (Wyeth), is a glycylcycline derivative of minocycline, a semi-synthetic tetracycline, with a 9-t-butylglycylamido substitution [1]. Tigecycline inhibits protein synthesis by binding to the 30S ribosomal subunit. Although it's antibacterial activity is significant, it is perceived to be bacteriostatic [2]. Tigecycline was developed to provide activity against tetracycline- and multi-drug-resistant gram-positive pathogens and has demonstrated significant broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative microorganisms [2-5].

Tigecycline resistance is very infrequent and is also difficult to induce in the laboratory [6, 7] with a selection frequency observed at less than 10^{-9} [3, 6, 8]. With the exception of *P. aeruginosa*, tetracycline-resistant bacteria with either tetracycline efflux pumps or ribosomal protective features are sensitive to tigecycline [2-4, 8-12]. The MIC_{90} values for pseudomonal isolates are generally elevated, in the range of 8-16 mcg/ml [11, 13]. The pharmacokinetics of parenteral tigecycline is linear with a mean half-life of 36 hours and a maximum serum concentration (C_{MAX}) of a 300mg dose infused over 1 hour of 2.8 mcg/ml [14, 15].

Since tigecycline has been shown to have activity against most members of non-Enterobacteriaceae, this study compared the activity of tigecycline with other agents against *Acinetobacter* spp and *Pseudomonas aeruginosa* from hospitals throughout Europe, the Middle East and South Africa.

MATERIALS & METHODS

- All isolates were derived from blood, respiratory tract, urine (no more than 30% of all isolates), skin, wound, fluids and other defined sources. Only one isolate per patient was accepted.
- Clinical isolates were collected between January 2001 - September 2002 from 36 study centers in 16 countries.
- Antimicrobial agents tested with concentrations (expressed in µg/mL) were: amoxicillin/clavulanic acid (0.5 - 64); ampicillin/sulbactam (0.5 - 32); cefepime (0.5 - 64); ceftazidime (0.5 - 64); ceftriaxone (0.5 - 64); doxycycline (0.06 - 32); imipenem (0.5 - 64); levofloxacin (0.25 - 64); tigecycline (0.008 - 16). MIC interpretive criteria followed published guidelines established by the NCCLS [13].
- Isolates were identified to genus and species at each site and confirmed by the central laboratory (Laboratories International for Microbiology Studies, Schaumburg, IL, USA).
- Organism collection, transport, confirmation of organism identification, antimicrobial susceptibility testing, as well as, development and management of a centralized database was coordinated by Laboratories International for Microbiology Studies (LIMS).

Antimicrobial Susceptibility Testing

- MIC's were determined by the central laboratory using broth microdilution panels manufactured by Dade Microscan (Dade Behring Inc., Sacramento, CA. USA) according to NCCLS guidelines and manufacturers instructions.
- Quality Control was performed using the following ATCC strains: *E. coli* ATCC 35218, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, and *S. aureus* ATCC 29213.

RESULTS

Results are shown in the following tables.

Table 2. In Vitro Activity (µg/mL) of Tigecycline and Comparative Agents Against 776 Isolates of *Acinetobacter* spp and *Pseudomonas aeruginosa*.

Organism / Phenotype	Drug	NCCLS BP*	Range	MIC_{50}	MIC_{90}	% Sus
			MIC (mcg/ml)			
Acinetobacter spp (470)	Tigecycline	n/a	0.12 - 4	0.5	2	--
	Doxycycline	4	0.06 - >32	0.25	>32	82.6
	Amox/Clav	n/a	0.25 - >64	16	>64	--
	Amp/Sulb	8/4	0.5 - >32	4	>32	71.1
	Cefepime	8	0.5 - >64	8	>64	55.1
	Ceftazidime	8	0.5 - >64	8	>64	54.9
	Ceftriaxone	8	0.5 - >64	16	>64	32.8
	Imipenem	4	0.5 - >64	0.5	32	83.8
	Levofloxacin	2	0.25 - >64	0.5	16	56.8
P. aeruginosa (306)	Tigecycline	n/a	1 - >16	16	>16	--
	Doxycycline	4	0.5 - >32	32	>32	1.3
	Amox/Clav	n/a	32 - >64	>64	>64	--
	Amp/Sulb	8/4	16 - >32	>32	>32	0.0
	Cefepime	8	0.5 - >64	4	16	85.0
	Ceftazidime	8	0.5 - >64	2	16	84.0
	Ceftriaxone	8	4 - >64	32	>64	15.0
	Imipenem	4	0.5 - >64	1	16	84.0
	Levofloxacin	2	0.25 - >64	0.5	16	74.8

n/a – Not Available. NCCLS breakpoints are undefined.

Table 3. In Vitro MICs (µg/mL) and Cumulative Percent Inhibited (%) of Tigecycline and Comparative Agents Against 470 Isolates of *Acinetobacter* spp

Antimicrobial (n/Cum%)	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
	MIC (mcg/ml)											
Tigecycline		15	142	127	126	56	4					
		3.2	33.4	60.4	87.2	99.1	100.0					
Doxycycline	65	131	53	44	60	24	11	9	18	51	4	
	13.8	41.7	53.0	62.3	75.1	80.2	82.6	84.5	88.3	99.1	100.0	
Amox/Clav			8	5	14	14	28	89	77	72	56	107
			1.7	2.8	5.7	8.7	14.7	33.6	50.0	65.3	77.2	100.0
Amp/Sulb				19	46	124	74	71	34	36	66	
				4.0	13.8	40.2	56.0	71.1	78.3	86.0	100.0	
Cefepime				35	37	85	61	41	62	54	46	49
				7.4	15.3	33.4	46.4	55.1	68.3	79.8	89.6	100.0
Ceftazidime				16	22	91	79	50	40	31	15	126
				3.4	8.1	27.4	44.3	54.9	63.4	70.0	73.2	100.0
Ceftriaxone				8	9	13	37	87	86	28	10	192
				1.7	3.6	6.4	14.3	32.8	51.1	57.0	59.1	100.0
Imipenem				300	40	34	20	20	8	2	4	42
				63.8	72.3	79.6	83.8	88.1	89.8	90.2	91.1	100.0
Levofloxacin			234	12	9	12	23	120	50	4	1	5
			49.8	52.3	54.3	56.8	61.7	87.2	97.9	98.7	98.9	100.0

Dividing lines represent MIC_{90} demarcation.

Table 4. In Vitro MICs (µg/mL) and Cumulative Percent Inhibited (%) of Tigecycline and Comparative Agents Against 307 Isolates of *Pseudomonas aeruginosa*

Antimicrobial (n/Cum%)	0.5	1	2	4	8	16	>16	32	>32	64	>64
	MIC (mcg/ml										
Tigecycline		4		14	88	142	59				
		1.3		5.9	34.5	80.8	100.0				
Doxycycline	1			3	28	111		71	93		
	0.3			1.3	10.4	46.6		69.7	100.0		
Amox/Clav								2		8	297
								0.7		3.3	100.0
Amp/Sulb						1		4	302		
						0.3		1.6	100.0		
Cefepime	4	33	104	70	50	27		9		8	2
	1.3	12.1	45.9	68.7	85.0	93.8		96.7		99.3	100.0
Ceftazidime	5	106	103	27	17	21		11		11	6
	1.6	36.2	69.7	78.5	84.0	90.9		94.5		98.0	100.0
Ceftriaxone				12	34	79		63		42	77
				3.9	15.0	40.7		61.2		74.9	100.0
Imipenem	86	130	30	12	11	28		6		1	3
	28.0	70.4	80.1	84.0	87.6	96.7		98.7		99.0	100.0
Levofloxacin		26	140	36	28	23	17	9		11	
		8.5	54.1	65.8	74.9	82.4	87.9	90.9		94.5	

Dividing lines represent MIC_{90} demarcation.

CONCLUSIONS

- Tigecycline inhibited 99% of *Acinetobacter* spp at a MIC of 2 µg/mL which was the lowest MIC_{90} of all comparator study drugs.
- Tigecycline was less active against *Pseudomonas aeruginosa* with a MIC_{90} of >16 µg/mL.
- The in vitro activity of tigecycline in this Gram-negative non-Enterobacteriaceae population warrants further investigation.
- Additional studies into the mechanism of resistance of *Pseudomonas aeruginosa* against tigecycline would be of interest.

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