

Tigecycline Evaluation Surveillance Trial (T.E.S.T.) Program - *In Vitro* Antibacterial Activity against Selected Species of non-glucose fermenting Gram negative rods from Asia and the Pacific Rim

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REVISED ABSTRACT

Background: Non-glucose fermenting Gram-negative rods are known to be highly resistant in hospital settings and have always been a challenge for clinicians and hospital infection control. The degree or type of resistance may be due to several sophisticated mechanisms such as production of extended spectrum beta-lactamase, efflux pumps, altered membrane permeability therefore inactivating most classes of broad spectrum antimicrobials that are available for treatment. Tigecycline, the first member of a new class of antimicrobials (glycylcyclines), has been shown to have potent activity against most species of *Enterobacteriaceae* and selected species of non-fermenters. The T.E.S.T. program determined the *in vitro* activity of tigecycline compared to amikacin, ampicillin, imipenem, cefepime, ceftazidime, ceftriaxone, levofloxacin, minocycline, and piperacillin/tazobactam against members of *Acinetobacter* spp. and *Pseudomonas aeruginosa* collected from hospitals across Asia and the Pacific Rim. **Methods:** A total of 194 clinical isolates (117 *P. aeruginosa* and 77 *Acinetobacter* spp.) were collected throughout 2004 from 6 centers and minimum inhibitory concentration (MICs) were determined by broth microdilution panels and interpreted according to CLSI guidelines. **Results:** All of the anti-pseudomonal agents ceftazidime, imipenem, piperacillin/tazobactam, cefepime, amikacin, and levofloxacin presented good inhibitory activity against *P. aeruginosa* with susceptibility rates above 65%. Tigecycline had an MIC₉₀ of >16 mcg/mL against *P. aeruginosa*. *A. baumannii* had the following susceptibility rates against the broad spectrum agents: cefepime 65%; amikacin 69%; ceftazidime 72%; levofloxacin 83%; imipenem 86%; pip/tazo 86%; minocycline 93%. Tigecycline had the lowest MIC₅₀/MIC₉₀ (0.12/1 mcg/ml) against *A. baumannii*. Performance of tigecycline was unaffected against multidrug-resistant isolates of *A. baumannii*. **Conclusion:** The presented data suggest that tigecycline may be an effective therapeutic option against multidrug-resistant nosocomial *Acinetobacter* spp. but ineffective against *Pseudomonas aeruginosa*.

INTRODUCTION

Tigecycline is a novel antimicrobial with an expanded broad-spectrum of activity from a new class of compounds, glycylcyclines. Tigecycline inhibits protein synthesis by binding to the 30S ribosomal subunit. Although it is perceived to be bacteriostatic, its anti-bacterial activity is significant and has shown some bactericidal activity against key targeted pathogens [1,2]. 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 [2-4].

Tigecycline resistance is very infrequent and is also difficult to induce in the laboratory [5, 6] with a selection frequency observed at less than 10⁻⁹ [3, 5, 7]. With the exception of *P. aeruginosa*, tetracycline-resistant bacteria with either tetracycline efflux pumps or ribosomal protective features are sensitive to tigecycline [2-4, 7-11]. The MIC₉₀ values for pseudomonal isolates are generally elevated, in the range of 8-16 mcg/ml due to synergism between outer membrane impermeability and efflux mechanisms [10]. However, tigecycline has been shown to be highly effective against multi-drug resistant *Acinetobacter* spp., particularly *A. baumannii* that are commonly associated with serious nosocomial infections [5].

This study prospectively compared the *in vitro* activity of tigecycline with comparative antimicrobial agents against *Acinetobacter* spp. and *P. aeruginosa* from Australia, China, India, Pakistan, Philippines and Singapore..

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.
- Clinical isolates were collected tested between January 2004 – December 2004 from 6 study centers in Australia, China, India, Pakistan, Philippines and Singapore.

- Custom broth microdilution panels were supplied by MicroScan (Dade Behring, 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 Clinical and Laboratory Standards Institute where applicable [12]. Tigecycline tentative breakpoints (in units of mcg/mL) are defined as susceptible ≤ 2; intermediate = 4; and resistant ≥ 8.
- Isolates were identified to genus and species at each site by the local laboratory. Isolates were tested by the local laboratory.
- Quality control of broth microdilution panels followed manufacture's and CLSI guidelines using the following ATCC strains: *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.
- The collection and transporting 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).

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RESULTS

Table 1. *In vitro* activity of tigecycline and comparative agents against 194 strains of *P. aeruginosa* and *Acinetobacter* spp. combined

Organism (n)	Drug	MIC (mcg/mL)		
		MIC ₅₀	MIC ₉₀	Range
<i>P. aeruginosa</i> & <i>Acinetobacter</i> spp (n=194)	Tigecycline	8	16	0.03 / >16
	Amikacin	4	>64	≤0.5 / >64
	Amox-Clav	>32	>32	1 / >32
	Ampicillin	>32	>32	1 / >32
	Cefepime	8	>32	≤0.5 / >32
	Ceftazidime	≤8	>32	≤8 / >32
	Ceftriaxone	>64	>64	≤0.06 / >64
	Imipenem	1	16	0.25 / >16
	Levofloxacin	1	>8	0.03 / >8
	Minocycline	16	>16	≤0.5 / >16
	Pip-Tazo	4	>128	≤0.06 / >128

Table 2. *In vitro* activity of tigecycline and comparative agents against 194 Gram-negative non-fermenters.

Organism (n)	Drug ^a	MIC (mcg/mL)				%Sus
		Range	MIC ₅₀	MIC ₉₀		
<i>Acinetobacter</i> spp (n=77)	Tigecycline	0.03 / 4	0.25	1	97.4	
	Amikacin	≤0.5 / >64	8	>64	63.6	
	Amox-Clav	1 / >32	16	>32	na	
	Ampicillin	1 / >32	>32	>32	na	
	Cefepime	≤0.5 / >32	16	>32	48.1	
	Ceftazidime	≤8 / >32	16	>32	49.4	
	Ceftriaxone	1 / >64	32	>64	32.5	
	Imipenem	0.25 / >16	0.5	>16	70.1	
	Levofloxacin	0.03 / >8	2	8	59.7	
	Minocycline	≤0.5 / 8	≤0.5	4	96.1	
	Pip-Tazo	≤0.06 / >128	4	>128	68.8	
	<i>Acinetobacter anitratus</i> (n=1)	Tigecycline	0.25 / 0.25	†	†	†
Amikacin		8 / 8	†	†	†	
Amox-Clav		32 / 32	†	†	†	
Ampicillin		>32 / >32	†	†	†	
Cefepime		2 / 2	†	†	†	
Ceftazidime		≤8 / =8	†	†	†	
Ceftriaxone		1 / 1	†	†	†	
Imipenem		1 / 1	†	†	†	
Levofloxacin		0.5 / 0.5	†	†	†	
Minocycline		1 / 1	†	†	†	
Pip-Tazo		0.25 / 0.25	†	†	†	
<i>Acinetobacter baumannii</i> (n=74)		Tigecycline	0.03 / 4	0.25	1	97.3
	Amikacin	≤0.5 / >64	8	>64	62.2	
	Amox-Clav	1 / >32	32	>32	na	
	Ampicillin	1 / >32	>32	>32	na	
	Cefepime	≤0.5 / >32	16	>32	45.9	
	Ceftazidime	≤8 / >32	16	>32	47.3	
	Ceftriaxone	1 / >64	32	>64	29.7	
	Imipenem	0.25 / >16	0.5	>16	68.9	
	Levofloxacin	0.03 / >8	2	8	58.1	
	Minocycline	≤0.5 / 8	≤0.5	4	95.9	
	Pip-Tazo	≤0.06 / >128	4	>128	67.6	
	<i>Acinetobacter lwoffii</i> (n=2)	Tigecycline	0.06 / 0.12	†	†	†
Amikacin		≤0.5 / 1	†	†	†	
Amox-Clav		1 / 4	†	†	†	
Ampicillin		1 / 4	†	†	†	
Cefepime		≤0.5 / 1	†	†	†	
Ceftazidime		≤8 / ≤8	†	†	†	
Ceftriaxone		1 / 4	†	†	†	
Imipenem		0.25 / 0.25	†	†	†	
Levofloxacin	0.12 / 0.12	†	†	†		

Organism (n)	Drug	MIC (mcg/mL)				
		≤0.5 / <0.5	†	†	†	†
<i>Pseudomonas aeruginosa</i> (n=117)	Pip-Tazo	≤0.06 / <0.06	†	†	†	†
	Tigecycline	0.5 / >16	8	>16	4.3	
	Amikacin	≤0.5 / >64	4	32	85.5	
	Amox-Clav	32 / >32	>32	>32	na	
	Ampicillin	16 / >32	>32	>32	na	
	Cefepime	≤0.5 / >32	8	>32	71.8	
	Ceftazidime	≤8 / >32	≤8	>32	70.9	
	Ceftriaxone	≤0.06 / >64	>64	>64	10.3	
	Imipenem	0.5 / >16	1	16	86.3	
	Levofloxacin	0.03 / >8	1	>8	62.4	
	Minocycline	2 / >16	>16	>16	3.4	
	Pip-Tazo	0.12 / >128	4	128	88.9	

^a Breakpoints as defined by CLSI where available (M100-S14), 2004; na = not available when breakpoints are undefined by the CLSI. Tigecycline breakpoints defined as: susceptible ≤2; intermediate = 4; and resistant ≥8. † MIC₅₀, MIC₉₀, and %Sus are not calculated for n's < 20.

Table 3. *In vitro* activity of tigecycline and comparators against 77 *Acinetobacter* spp. showing cumulative percent inhibited (%) at each MIC (mcg/ml)

n/Clum%	MIC (mcg/mL) ^a																
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128			
Tigecycline	4	14	20	12	19	6	2	2	8	16	32	64	128	>128			
Amikacin	5.2	23.4	49.4	64.9	89.6	97.4	100										
Amox-Clav					2	5	20	11	8	3	4	24					
Ampicillin					2.6	9.1	35.1	49.4	59.7	63.6	2	68.8	100				
Cefepime					2	2	1	11	10	15	2	36					
Ceftazidime					2.6	2.6	3.9	18.2	31.2	50.6	53.2	100					
Ceftriaxone					6	7	12	10	2	8	9	23					
Imipenem					7.8	16.9	32.5	45.5	48.1	58.4	70.1	100					
Levofloxacin									38	3	1	35					
Minocycline									49.4	53.2	54.5	100					
Pip-Tazo									6	13	10	6	2	34			
									32.5	45.5	55.2	55.8	100				
					28	12	12	7.8	15.6	32.5	45.5	55.2	55.8	100			
					36.4	51.9	67.5	70.1	72.7	85.7	100						
					2	1	2	9	17	9	5						
					45.5	48.1	59.7	81.8	93.5	100							
					42	12	10	10	3	1							
					54.5	70.1	83.1	96.1	100								
					1	2	4	5	1	3	4	5	1	23			
					20.8	31.2	36.4	37.7	40.3	45.5	51.9	53.2	57.1	62.3	68.8	70.1	100

* Some ≤ and > values have been normalized by rounding up to the next highest MIC.

Table 4. *In vitro* activity of tigecycline and comparators against 117 *Pseudomonas aeruginosa* showing cumulative percent inhibited (%) at each MIC (mcg/ml)

n/Clum%	MIC (mcg/mL) ^a															
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128		
Tigecycline					1.7	3.4	4.3	14.5	60.7	89.7	100					
Amikacin					1	5	33	40	19	2	7	7	3			
Amox-Clav					0.9	5.1	33.3	67.5	83.8	85.5	91.5	97.4	100			
Ampicillin											8	109				
Cefepime					2	4	37	15	26	12	6	15				
Ceftazidime					1.7	5.1	36.8	49.6	71.8	82.1	87.2	100				
Ceftriaxone									83	6	6	22				
Imipenem									70.9	76.1	81.2	100				
Levofloxacin					1	2	2	2	6	8	11	9	77			
Minocycline					0.9				11.7	3.4	5.1	10.3	17.1	26.5	34.2	100
Pip-Tazo					23	63	7	8	4							