In Vitro Study to Determine the Activity of Tigecycline against Fastidious Isolates from the Tigecycline Evaluation Surveillance Trial (T.E.S.T.) Program from Asia and the Pacific Rim

 # ST13

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REVISED ABSTRACT

Objective: Tigecycline is a novel glycylcycline, which has been shown to have potent activity against organisms with either ribosomal protection or active efflux. Tigecycline has shown excellent *in vitro* activity against fastidious isolates. The T.E.S.T. program determined the activity of tigecycline as compared to those of comparative agents against *Streptococcus pneumoniae*, *Streptococcus agalactiae* and *Haemophilus influenzae* from hospital-based investigative centers in Asia. **Methods:** A total of 191 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Isolates were collected throughout 2004 from centers across Asia and the Pacific Rim. MIC's of tigecycline and comparator antimicrobial agents were determined by the local laboratory using broth microdilution panels from Dade MicroScan according to CLSI guidelines and manufacturer's instructions. **Results:** Tigecycline had a MIC_{90} of ≤ 0.5 mcg/mL against all the fastidious organisms tested. Of *S. pneumoniae*, 30.7% were non-susceptible to penicillin (I+R). Twenty one percent (21%) of *H. influenzae* were ß-lactamase producers. Tigecycline had a MIC_{90} of 0.5 mcg/ml against all *S. pneumoniae*, 2 mcg/ml against *S. agalactiae*, and 0.12 mcg/ml against ß-lactamase positive *H. influenzae*. **Conclusion:** Tigecycline's activity is comparable to all of most commonly prescribed and broad spectrum antimicrobial agents evaluated in this study. The results indicate that tigecycline is an effective *in vitro* against the fastidious isolates, regardless of penicillin susceptibility or ß-lactamase production. Tigecycline may serve as an effective alternative therapeutic option for infections caused by fastidious species.

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 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, 7-9]. Tigecycline has demonstrated MIC_{90} values of ≤0.5 mcg/mL against *Streptococcus pneumoniae*, *Streptococcus pyogenes* and other Gram-positive organisms [2, 4-6]. Tigecycline has shown potent activity against non-enterobacteriaceae Gram-negative aerobes such as *Haemophilus influenzae* with MIC_{90}s of 0.25 mcg/mL regardless of beta-lactamase activity.

This study was designed to better define the *in vitro* activity of tigecycline in a limited number of fastidious clinical isolates collected from 6 study centers in Australia, China, India, Pakistan, Philippines and Singapore.

MATERIALS & METHODS

- All isolates were derived from blood, genitourinary, respiratory tract and other sources. Only one isolate per patient was accepted.
- There were 191 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 MicroScan, 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 [11]. 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 by the local laboratory. Each site tested the isolates using broth microdilution.
- Quality control of broth microdilution panels followed manufacture's and CLSI guidelines using the following ATCC strains: *Haemophilus influenzae* ATCC 49247; *Haemophilus influenzae* ATCC 49766; and *Streptococcus pneumoniae* ATCC 49619.
- 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, USA).

ACKNOWLEDGEMENTS

This study was supported by a grant from Wyeth Pharmaceuticals. We gratefully acknowledge contributions to the T.E.S.T. program from the following participating institutions: P.D. Hinduja National Hospital, India; Research Institute for Tropical Medicine, Philippines and Peking Union Medical College Hospital, China; Singapore General Hospital, Singapore; Aga Khan University & Hospital, Pakistan; Flinders Medical Center, Philippines.

REFERENCES

1 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.
2 Abbanat, D., M. Macielag, and K. Bush, *Novel antibacterial agents for the treatment of serious Gram-positive infections.* Expert Opin Investig Drugs, 2003. **12**(3): p. 379-99.
3 Betriu, 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.
4 Gales, 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.
5 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.
6 Chopra, I., *New developments in tetracycline antibiotics: glycylcyclines and tetracycline efflux pump inhibitors.* Drug Resist Updat, 2002. **5**(3-4): p. 119-25.
7 Projan, S.J., *Preclinical pharmacology of GAR-936, a novel glycylcycline antibacterial agent.* Pharmacotherapy, 2000. **20**(9 Pt 2): p. 219S-223S; discussion 224S-228S.
8 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.
9 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.
10 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. 2595-601.
11 Clinical Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement. CLSI document M100-S14. Wayne, PA, 2004.

RESULTS

Table 1. *In vitro* Activity of Tigecycline and 10 Comparators against 71 Strains of *Haemophilus influenzae* from Asia and the Pacific Rim Characterized by Beta-lactamase Activity.

Organism (n)	Drug [a]	%Sus	%Int	%Res	MIC (mcg/mL) MIC_{50}	MIC_{90}	Range
H. influenzae	Tigecycline	100.0	0.0	0.0	0.12	0.25	≤0.008 / 0.25
(n=71)	Amikacin	na	na	na	8	8	≤0.5 / 16
	Amox-Clav	100.0	0.0	0.0	0.5	1	≤0.12 / 2
	Ampicillin	77.5	8.5	14.1	≤0.5	16	≤0.5 / >32
	Cefepime	98.6	0.0	1.4	≤0.5	≤0.5	≤0.5 / 4
	Ceftazidime	0.0	0.0	100.0	≤8	≤8	≤8 / 16
	Ceftriaxone	100.0	0.0	0.0	≤0.06	≤0.06	≤0.06 / 1
	Imipenem	98.6	0.0	1.4	0.5	1	≤0.06 / 8
	Levofloxacin	98.6	0.0	1.4	0.015	0.12	≤0.008 / 8
	Minocycline	na	na	na	≤0.5	1	≤0.5 / 1
	Pip-Tazo	100.0	0.0	0.0	≤0.06	0.12	≤0.06 / 0.5
Beta-lactamase	Tigecycline	100.0	0.0	0.0	0.12	0.25	0.015 / 0.25
Negative	Amikacin	na	na	na	8	8	≤0.5 / 16
H. influenzae	Amox-Clav	100.0	0.0	0.0	0.5	1	≤0.12 / 2
(n=56)	Ampicillin	94.6	5.4	0.0	≤0.5	1	≤0.5 / 2
	Cefepime	98.2	0.0	1.8	≤0.5	≤0.5	≤0.5 / 4
	Ceftazidime	0.0	0.0	100.0	≤8	≤8	≤8 / 16
	Ceftriaxone	100.0	0.0	0.0	≤0.06	0.25	≤0.06 / 1
	Imipenem	98.2	0.0	1.8	0.5	1	0.12 / 8
	Levofloxacin	98.2	0.0	1.8	0.015	0.25	≤0.008 / 8
	Minocycline	na	na	na	≤0.5	1	≤0.5 / 1
	Pip-Tazo	100.0	0.0	0.0	≤0.06	0.12	≤0.06 / 0.5
Beta-lactamase	Tigecycline	100.0	0.0	0.0	0.06	0.12	≤0.008 / 0.25
Positive	Amikacin	na	na	na	4	8	≤0.5 / 8
H. influenzae	Amox-Clav	100.0	0.0	0.0	1	1	≤0.12 / 2
(n=15)	Ampicillin	13.3	20.0	66.7	16	>32	≤0.5 / >32
	Cefepime	100.0	0.0	0.0	≤0.5	≤0.5	≤0.5 / =0.5
	Ceftazidime	0.0	0.0	100.0	≤8	≤8	≤8 / ≤8
	Ceftriaxone	100.0	0.0	0.0	≤0.06	≤0.06	≤0.06 / ≤0.06
	Imipenem	100.0	0.0	0.0	0.5	1	≤0.06 / 1
	Levofloxacin	100.0	0.0	0.0	0.015	0.015	≤0.008 / 0.5
	Minocycline	na	na	na	≤0.5	1	≤0.5 / 1
	Pip-Tazo	100.0	0.0	0.0	≤0.06	≤0.06	≤0.06 / ≤0.06
Penicillin-Intermediate	Tigecycline	100.0	0.0	0.0	0.5	0.5	0.03 / 0.5
S. pneumoniae	Amox-Clav	100.0	0.0	0.0	0.25	1	≤0.03 / 1
(n=19)	Ampicillin	na	na	na	0.25	2	≤0.06 / 2
	Ceftriaxone	100.0	0.0	0.0	0.12	1	≤0.03 / 1
	Imipenem	15.8	84.2	0.0	0.25	0.5	≤0.12 / 0.5
	Levofloxacin	100.0	0.0	0.0	0.5	1	0.5 / 2
	Linezolid	100.0	0.0	0.0	≤0.5	1	≤0.5 / 1
	Minocycline	na	na	na	4	>8	≤0.25 / >8
	Penicillin	0.0	100.0	0.0	0.25	1	0.12 / 1
	Pip-Tazo	na	na	na	≤0.25	2	≤0.25 / 2
	Vancomycin	94.7	0.0	5.3	0.25	1	0.25 / 2
Penicillin-Resistant	Tigecycline	100.0	0.0	0.0	0.5	0.5	0.12 / 0.5
S. pneumoniae	Amox-Clav	83.3	16.7	0.0	1	4	≤0.03 / 4
(n=6)	Ampicillin	na	na	na	2	>16	1 / >16
	Ceftriaxone	100.0	0.0	0.0	0.5	1	≤0.03 / 1
	Imipenem	0.0	83.3	16.7	0.25	2	0.25 / 2
	Levofloxacin	66.7	16.7	16.7	1	8	0.5 / 8
	Linezolid	100.0	0.0	0.0	≤0.5	2	≤0.5 / 2
	Minocycline	na	na	na	4	>8	1 / >8
	Penicillin	0.0	0.0	100.0	2	>8	2 / >8
	Pip-Tazo	na	na	na	2	8	≤0.25 / 8
	Vancomycin	83.3	0.0	16.7	0.25	2	0.25 / 2
S. agalactiae	Tigecycline	100.0	0.0	0.0	0.12	2	≤0.008 / 2
(n=39)	Amox-Clav	na	na	na	0.06	0.5	≤0.03 / 1
	Ampicillin	94.9	0.0	5.1	0.12	0.25	≤0.06 / 1
	Ceftriaxone	92.3	0.0	7.7	0.06	0.25	≤0.03 / >64
	Imipenem	na	na	na	0.25	0.5	≤0.12 / 1
	Levofloxacin	87.2	0.0	12.8	0.5	8	0.5 / 16
	Linezolid	100.0	0.0	0.0	1	1	≤0.5 / 2
	Minocycline	na	na	na	8	>8	≤0.25 / >8
	Penicillin	94.9	0.0	5.1	≤0.06	0.12	≤0.06 / 4
	Pip-Tazo	na	na	na	≤0.25	0.5	≤0.25 / 4
	Vancomycin	100.0	0.0	0.0	0.5	0.5	0.25 / 1

[a] Breakpoints as defined by CLSI where available (M100-S14), 2004. na = CLSI breakpoints not available. Tigecycline breakpoints defined as: susceptible ≤2; intermediate = 4; and resistant ≥8.

Table 2. Frequency Distribution and Cumulative Percents Inhibited for Tigecycline and Comparative Agents against 71 Strains of *Haemophilus Influenzae*.

	MIC (mcg/mL)												
n/Cum%	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
Tigecycline	1	3	1	15	36	15								
	1.4	5.6	7.0	28.2	78.9	100.0								
Amikacin									3	3	4	19	37	5
									4.2	8.5	14.1	40.8	93.0	100.0
Amox-Clav							6	16	34	12	3			
							8.5	31.0	78.9	95.8	100.0			
Ampicillin									52	3	6	1	1	2	4	2
									73.2	77.5	85.9	87.3	88.7	91.5	97.2	100.0
Cefepime									68		2	1				
									95.8		98.6	100.0				
Ceftazidime													70	1
													98.6	100.0
Ceftriaxone						64	1	1	3	2				
						90.1	91.5	93.0	97.2	100.0				
Imipenem						2	1	9	37	18	3		1	
						2.8	4.2	16.9	69.0	94.4	98.6		100.0	
Levofloxacin	13	47	1	1	2	2	1	3					1	
	18.3	84.5	85.9	87.3	90.1	93.0	94.4	98.6					100.0	
Minocycline							58	13						
							81.7	100.0						
Pip-Tazo				61	6	2	2							
				85.9	94.4	97.2	100.0							

Table 3. *In vitro* Activity of Tigecycline and 10 Comparators against 120 Strains of *Streptococcus pneumoniae* and *Streptococcus agalactiae* from Asia and the Pacific Rim

Organism (n)	Drug [a]	%Sus	%Int	%Res	MIC (mcg/mL) MIC_{50}	MIC_{90}	Range
S. pneumoniae	Tigecycline	100.0	0.0	0.0	0.5	0.5	≤0.008 / 0.5
(n=81)	Amox-Clav	98.8	1.2	0.0	≤0.03	0.5	≤0.03 / 4
	Ampicillin	na	na	na	≤0.06	1	≤0.06 / >16
	Ceftriaxone	100.0	0.0	0.0	≤0.03	0.5	≤0.03 / 1
	Imipenem	69.1	29.6	1.2	≤0.12	0.25	≤0.12 / 2
	Levofloxacin	97.5	1.2	1.2	0.5	1	≤0.06 / 8
	Linezolid	100.0	0.0	0.0	≤0.5	1	≤0.5 / 2
	Minocycline	na	na	na	1	8	≤0.25 / >8
	Penicillin	69.1	23.5	7.4	≤0.06	1	≤0.06 / >8
	Pip-Tazo	na	na	na	≤0.25	1	≤0.25 / 8
	Vancomycin	97.5	0.0	2.5	0.25	0.5	≤0.12 / 2
S. pneumoniae	Tigecycline	100.0	0.0	0.0	0.5	0.5	≤0.008 / 0.5
Penicillin-Susceptible	Amox-Clav	100.0	0.0	0.0	≤0.03	≤0.03	≤0.03 / 0.06
(n=56)	Ampicillin	na	na	na	≤0.06	≤0.06	≤0.06 / 0.12
	Ceftriaxone	100.0	0.0	0.0	≤0.03	≤0.03	≤0.03 / 0.12
	Imipenem	94.6	5.4	0.0	≤0.12	≤0.12	≤0.12 / 0.25
	Levofloxacin	100.0	0.0	0.0	0.5	1	≤0.06 / 1
	Linezolid	100.0	0.0	0.0	≤0.5	1	≤0.5 / 1
	Minocycline	na	na	na	≤0.25	8	≤0.25 / >8
	Penicillin	100.0	0.0	0.0	≤0.06	≤0.06	≤0.06 / ≤0.06
	Pip-Tazo	na	na	na	≤0.25	≤0.25	≤0.25 / ≤0.25
	Vancomycin	100.0	0.0	0.0	0.25	0.5	≤0.12 / 0.5

Table 4. Frequency Distribution and Cumulative Percents Inhibited for Tigecycline and Comparative Agents against 81 Strains of *Streptococcus pneumoniae*.

	MIC (mcg/mL)											
n/Cum%	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
Tigecycline	3	6	3	2	4	41	22						
	3.7	11.1	14.8	17.3	22.2	39.5	100.0						
Amox-Clav				55	5	6	5	3	4	2	1		
				67.9	74.1	81.5	87.7	91.4	96.3	98.8	100.0		
Ampicillin					57	6	4	3	4	4	2		1
					70.4	77.8	82.7	86.4	91.4	96.3	98.8		100.0
Ceftriaxone				55	8	5	4	4	5				
				67.9	77.8	84.0	88.9	93.8	100.0				
Imipenem					56	20	4		1				
					69.1	93.8	98.8		100.0				
Levofloxacin				1	1	4	39	33	1	1	1		
				1.2	2.5	7.4	55.6	96.3	97.5	98.8	100.0		
Linezolid							48	32	1				
							59.3	98.8	100.0				
Minocycline						34	2	6	9	12	11	7	
						42.0	44.4	51.9	63.0	77.8	91.4	100.0	
Penicillin				56	7	4	3	5	5			1	
				69.1	77.8	82.7	86.4	92.6	98.8			100.0	
Pip-Tazo					69			4	5	2	1		
						85.2		90.1	96.3	98.8	100.0		
Vancomycin					3	51	24	1	2				
					3.7	66.7	96.3	97.5	100.0				

Table 5. Frequency Distribution and Cumulative Percents Inhibited for Tigecycline and Comparative Agents against 39 Strains of *Streptococcus agalactiae*.

	MIC (mcg/mL)											
n/Cum%	≤0.008	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>64
Tigecycline	1	3	10	7			14	4				
	2.6	10.3	35.9	53.8			89.7	100				
Amox-Clav		5	18	11	1	3	1					
		12.8	59	87.2	89.7	97.4	100					
Ampicillin			11	19	7		2					
			28.2	76.9	94.9		100					
Ceftriaxone		5	18	12	1					1		2
		12.8	59	89.7	92.3					94.9		100
Imipenem			16	3	19	3	1					
			41	89.7	97.4	100						
Levofloxacin				23	11						2	3
				59	87.2						92.3	100
Linezolid				3	3	1						
				7.7	97.4	100						
Minocycline				9				1	1	11	17	
				23.1				25.6	28.2	56.4	100	
Penicillin		26	11					1	1			
		66.7	94.9					97.4	100			
Pip-Tazo				33	3	1	1	1	1			
				84.6	92.3	94.9	97.4	100				
Vancomycin				8	28	3						
				20.5	92.3	100						

CONCLUSIONS

- Tigecycline inhibited the growth of all strains of *H. influenzae* at a MIC ≤0.25 mcg/ml without regard to beta-lactamase production.
- Tigecycline inhibited all strains of *S. pneumoniae* at MICs ≤0.5 mcg/mL without regard to penicillin sensitivity.
- Tigecycline demonstrates *in vitro* activity comparable to or greater than commonly prescribed antimicrobial agents such as linezolid, ceftriaxone, levofloxacin and amoxicillin-clavulanic acid currently used for the treatment of serious infections caused by fastidious Gram-negative and Gram-positive pathogens including penicillin-resistant *S. pneumoniae* and beta-lactamase producing strains of *H. influenzae*.