In Vitro Study to Determine the Activity of Tigecycline as Compared to Nine Comparator Antimicrobials Against Various Fastidious Isolates from the Tigecycline Evaluation Surveillance Trial (T.E.S.T.)

#FP-G-16

B. Johnson[1], T. Stevens[1], S. Bouchillon[1], J. Johnson[1], D. Hoban[1], M. Person[1], M. McCarthy[1], M. Dowzicky[2]

[1]International Health Management Associates, Schaumburg, IL, USA
[2]Wyeth Pharmaceuticals, Collegeville, PA, USA

IHMA, Inc.
2122 Palmer Dr.
Schaumburg, IL 60173
Tel: (847) 303-5003
Fax: (847) 303-5601
www.ihmainc.com

REVISED ABSTRACT

Background: Tigecycline (GAR-936) is a new 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. determined the activity of tigecycline as compared to those of comparative agents against *Streptococcus pneumoniae*, *Streptococcus agalactiae* and *Haemophilus influenzae* from hospitals-based investigative centers worldwide. **Methods:** A total of 866 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Isolates were collected between January 2004 – November 2004. MIC's of tigecycline and several comparator antimicrobial agents were determined by the local laboratory using broth microdilution panels from Dade Microscan according to NCCLS guidelines and manufacturer's instructions. **Results:** Tigecycline had a MIC_{90} of ≤0.5 mcg/mL against all the fastidious organisms tested with a MIC_{90} of 0.5 mcg/ml against both penicillin susceptible *S. pneumoniae* (PSSP), and penicillin resistant *S. pneumoniae* (PRSP), 0.25 mcg/ml against *S. agalactiae*, 0.25 mcg/ml against both beta-lactamase negative and positive *H. influenzae*. **Conclusion:** Tigecycline's activity is comparable or superior to all of most commonly prescribed and broad spectrum antimicrobial agents evaluated in this study. The results show Tigecycline as an effective antimicrobial agent against the selected fastidious isolates with lower or equivalent MICs against all fastidious strains in this study, including PRSP.

INTRODUCTION

In recent years there has been a dramatic rise in resistance to commonly used antimicrobial agents in the treatment of both community acquired and nosocomial infections. Many of these strains have developed resistance to two or more antibiotics agent curtailing the use of entire genres of antimicrobials. Some countries are reporting more than 50% resistance of *S. pneumoniae* to penicillin and macrolides [1].

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 MIC^{90} values of ≤0.5 mcg/ml have been demonstrated against methicillin-resistant and multi-drug resistant *Staphylococcus aureus* and *Streptococcus pneumoniae* [3-5].

Tigecycline resistance is very infrequent and difficult to induce in the laboratory with a selection frequency observed at less than 10^{-9} [6, 7]. Most tetracycline-resistant bacteria with either tetracycline efflux pumps or ribosomal protective features are sensitive to tigecycline.

This study compared the activity of tigecycline with other agents against clinical isolates of three fastidious species, *S. pneumoniae,* with penicillin non-susceptible phenotypes, *S. agalactiae*, and *H. influenzae*, with beta-lactamase producing strains from geographically diverse institutions in North America and Europe.

MATERIALS & METHODS

- All isolates were derived from blood and respiratory tract sources. Only one isolate per patient was accepted.
- Clinical isolates were collected tested between January 2004 - November 2004 from 20 study centers in 6 countries.
- Antimicrobial agents tested with concentrations (expressed in mcg/ml) were for Gram-positive isolates amoxicillin/clavulanic acid (0.03-8); piperacillin/tazobactam (0.25-16); levofloxacin (0.06-32); ceftriaxone (0.03-64); linezolid (0.5-8); minocycline (0.25-8); vancomycin (0.12-32); ampicillin (0.06-16); penicillin (0.06-8); tigecycline (0.008-16); imipenem (0.12-16) and Gram-negative isolates 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); imipenem (0.06-16). MIC interpretive criteria followed published guidelines established by the NCCLS where applicable [8]. 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.
- Organism collection, transport, confirmation of organism identification, as well as, construction and management of a centralized database was coordinated by Laboratories International for Microbiology Studies (LIMS).

REFERENCES

1 Schito, G. C., E. A. Debbia, and A. Marchese**.** 2000. The evolving threat of antibiotic resistance in Europe: new data from the Alexander Project. J. Antimicrob. Chemother. **46**(S-T1)**:**3-9
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. Milatovic, D., et al., *Activities of the Glycylcycline Tigecycline (GAR-936) against 1,924 Recent European Clinical Bacterial Isolates.* Antimicrob Agents Chemother, 2003. **47**(1): p. 400-4.
4. Petersen, P.J., et al., *In vitro and in vivo antibacterial activities of a novel glycylcycline, the 9-t-butylglycylamido derivative of minocycline (GAR-936).* Antimicrob Agents Chemother, 1999. **43**(4): p. 738-44.
5. Fritsche, T.R., J.T. Kirby, and R.N. Jones, *In vitro activity of tigecycline (GAR-936) tested against 11,859 recent clinical isolates associated with community-acquired respiratory tract and gram-positive cutaneous infections.* Diagn Microbiol Infect Dis, 2004. **49**(3): p. 201-9.
6. 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.
7. Chopra, I., *New developments in tetracycline antibiotics: glycylcyclines and tetracycline efflux pump inhibitors.* Drug Resist Updat, 2002. **5**(3-4): p. 119-25.
8. National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement. NCCLS document M100-S14. Wayne, PA, 2004.

ACKNOWLEDGEMENTS

This study was supported by a grant from Wyeth Pharmaceuticals.
We gratefully acknowledge Andre Hsiung for manuscript preparation.and the contributions to this study from the following participating institutions:

U.S.A., University of Florida; U.S.A., Jackson Memorial; U.S.A., University of Maryland Medical Center; U.S.A., University of Michigan Medical Center; U.S.A., William Beaumont Hospital; U.S.A., Montefiore Medical Center; U.S.A, Columbia Presbyterian Medical Center; U.S.A., Univ. of Rochester Medical Center; U.S.A., Summa Health System; U.S.A., University Hospitals of Cleveland; U.S.A., RML @ SJMC; U.S.A., Memorial Hermann Hospital; U.S.A., Clarian Health Partners, Inc./Methodist Hospital; Italy, Institute of Micro./Univ. of Ancona Med. School; Canada, Toronto Medical Labs/Mount Sinai Hospital; U.S.A., Cleveland Clinic Foundation; U.S.A., Inova Fairfax Hospital; Spain, Hospital General Universitario Gregorio Maranon; United Kingdom, Kings College; Germany, Institute for Med Microbiology

RESULTS

The results are published in the following tables.

Table 1. In vitro Activity (MIC mcg/mL) of Tigecycline and Comparative Agents against 321 *Streptococcus pneumoniae* and Penicillin-Susceptible, Intermediate and Resistant Phenotypes.

Organism Name	Drug	MIC (mcg/mL) MIC_{50}	MIC_{90}	Range	%SUS*
Streptococcus pneumoniae	Tigecycline	0.06	0.5	≤0.03/1	100.0
(n=321)	Amox/Clav	0.06	2	≤0.008/8	90.5
	Ampicillin	≤0.06	4	≤0.06/8	61.0
	Ceftriaxone	≤0.03	1	≤0.03/4	98.8
	Levofloxacin	0.5	1	≤0.06/32	97.8
	Linezolid	≤0.5	1	≤0.5/1	100.0
	Minocycline	≤0.25	4	≤0.25/>8	85.1
	Penicillin	≤0.06	2	≤0.06/>8	61.4
Penicillin-Susceptible	Tigecycline	0.06	0.5	≤0.008/1	100.0
Streptococcus pneumoniae	Amox/Clav	≤0.03	≤0.03	≤0.03/0.25	100.0
(n=197)	Ampicillin	≤0.06	≤0.06	≤0.06/0.12	100.0
	Ceftriaxone	≤0.03	≤0.03	≤0.03/0.12	100.0
	Levofloxacin	0.5	1	≤0.06/1	100.0
	Linezolid	≤0.5	1	≤0.5/1	100.0
	Minocycline	≤0.25	1	≤0.25/8	93.2
	Penicillin	≤0.06	≤0.06	≤0.06/≤0.0(	100.0
Penicillin-Intermediate	Tigecycline	0.06	0.25	≤0.008/1	100.0
Streptococcus pneumoniae	Amox/Clav	0.25	2	≤0.03/4	98.1
(n=73)	Ampicillin	0.5	2	≤0.06/4	100.0
	Ceftriaxone	0.25	0.5	≤0.03/1	100.0
	Levofloxacin	1	1	≤0.06/32	96.2
	Linezolid	≤0.5	1	≤0.5/2	100.0
	Minocycline	≤0.25	8	≤0.25/>8	84.6
	Penicillin	0.5	1	0.12/1	0.0
Penicillin-Resistant	Tigecycline	0.06	0.5	0.015/1	100.0
Streptococcus pneumoniae	Amox/Clav	4	8	0.12/8	47.6
(n=51)	Ampicillin	4	8	0.25/8	0.0
	Ceftriaxone	1	1	0.03/4	92.2
	Levofloxacin	1	1	0.25/8	96.1
	Linezolid	≤0.5	1	≤0.5/1	100.0
	Minocycline	2	8	≤0.25/>8	52.9
	Penicillin	2	8	2/>8	0.0

* Breakpoints defined by the NCCLS 2004, document M100-S14; Tigecycline and minocycline breakpoints (in mcg/mL) defined as susceptible ≤2; intermediate = 4; resistant ≤8; Ampicillin susceptibility defined according to the susceptibility or penicillin.

Table 2. *In vitro* Activity (MIC mcg/mL) of Tigecycline and Comparative Agents against 235 *Streptococcus agalactiae*.

Organism Name	Drug	MIC (mcg/mL) MIC_{50}	MIC_{90}	Range	%SUS*
Streptococcus agalactiae	Tigecycline	0.06	0.25	0.015/1	100.0
(n=235)	Amox/Clav	0.06	0.12	≤0.03/>8	93.3
	Ampicillin	0.12	0.25	≤0.06/>16	100.0
	Ceftriaxone	0.06	0.12	≤0.03/>64	100.0
	Levofloxacin	0.5	1	0.25/>32	95.3
	Linezolid	1	1	≤0.5/0.5	100.0
	Minocycline	8	>8	≤0.25/>8	14.0
	Penicillin	≤0.06	0.12	≤0.06/>8	100.0

* Breakpoints defined by the NCCLS 2004, document M100-S14; Tigecycline and minocycline breakpoints (in mcg/mL) defined as susceptible ≤2; intermediate = 4; resistant≤8; Amoxicillin-clavulanic acid susceptibility defined according to the susceptibility or penicillin.

Table 3. *In vitro* Activity (MIC mcg/mL) of Tigecycline and Comparative Agents against 310 *Haemophilus influenzae* and Beta-lactamase Positive and Negative Phenotypes.

Organism Name	Drug	MIC (mcg/mL) MIC_{50}	MIC_{90}	Range	%SUS*
Haemophilus influenzae	Tigecycline	0.12	0.25	≤0.008/1	100.0
(n=310)	Amox/Clav	0.5	1	≤0.12/4	100.0
	Ampicillin	≤0.5	>32	≤0.5/>32	74.8
	Cefepime	≤0.5	≤0.5	≤0.5/8	99.0
	Ceftazidime	≤8	≤8	≤8/16	0.4
	Ceftriaxone	≤0.06	≤0.06	≤0.06/0.5	100.0
	Imipenem	0.5	1	≤0.06/>16	99.4
	Levofloxacin	0.015	0.03	≤0.008/8	100.0
	Pip/Tazo	≤0.06	≤0.06	≤0.06/1	100.0
	Minocycline	≤0.5	1	≤0.5/>16	99.7
Beta-lactamase Positive	Tigecycline	0.12	0.25	≤0.008/1	100.0
Haemophilus influenzae	Amox/Clav	1	2	≤0.12/4	100.0
(n=80)	Ampicillin	32	>32	≤0.5/>32	6.3
	Ceftazidime	≤8	≤8	≤8/≤8	0.0
	Ceftriaxone	≤0.06	≤0.06	≤0.06/0.25	100.0
	Levofloxacin	0.015	0.015	≤0.008/0.5	100.0
	Minocycline	≤0.5	1	≤0.5/>2	100.0
	Pip/Tazo	≤0.06	≤0.06	≤0.06/0.5	100.0
	Cefepime	≤0.5	≤0.5	≤0.5/0.5	100.0
	Imipenem	0.5	1	0.12/4	100.0
Beta-lactamase Negative	Tigecycline	0.12	0.25	≤0.008/0.5	100.0
Haemophilus influenzae	Amox/Clav	0.5	1	≤0.12/4	100.0
(n=230)	Ampicillin	≤0.5	≤0.5	≤0.5/>32	98.7
	Ceftazidime	≤8	≤8	≤8/16	0.6
	Ceftriaxone	≤0.06	≤0.06	≤0.06/0.5	100.0
	Levofloxacin	0.015	0.03	≤0.008/1	100.0
	Minocycline	≤0.5	1	≤0.5/4	99.6
	Pip/Tazo	≤0.06	≤0.06	≤0.06/1	100.0
	Cefepime	≤0.5	≤0.5	≤0.5/8	98.7
	Imipenem	0.5	1	≤0.06/>16	99.1

* Breakpoints defined by the NCCLS 2004, document M100-S14; Tigecycline and minocycline breakpoints (in mcg/mL) defined as susceptible ≤2; intermediate = 4; resistant e" 8.

CONCLUSIONS

- Tigecycline's MIC_{90} of 0.5 mcg/mL was the lowest of all comparative agents against all strains of *Streptococcus pneumoniae*.
- Tigecycline's MIC_{90} of 0.5 mcg/mL against penicillin-resistant *Streptococcus pneumoniae* lower than broad spectrum ceftriaxone, levofloxacin, and linezolid and 8 fold lower than amoxicillin-clavulanic acid.
- Tigecycline inhibited 99.6% of all *Streptococcus agalactiae* at a MIC of 1 mcg/mL.
- Tigecycline had a MIC_{50} and MIC_{90} of 0.12 and 0.25 mcg/mL, respectively, against all strains of *Haemophilus influenzae* that was not affected by the production of beta-lactamase.
- Tigecycline is a promising potent antimicrobial agent against fastidious organisms.