445

A Global Surveillance to Determine Tigecycline's In Vitro Activity against Multiple Resistant Phenotypes of Enterobacteriaceae

B. Johnson[1], S. Bouchillon[1], T. Stevens[1], J. Johnson[1], D. Hoban[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, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections including GN, GP, Anaerobic and resistant strains. The T.E.S.T. program determined the in vitro activity of tigecycline compared to amoxicillin-clavulanic acid, piperacillin-tazobactam, levofloxacin, ceftriaxone, cefepime, ampicillin, amikacin, minocycline, ceftazidime and imipenem against *Enterobacteriaceae* species collected from hospitals globally throughout 2004-2005. The objective of this study was to evaluate the activity of tigecycline against resistant *Enterobacteriaceae* phenotypes commonly associated with nosocomial infections. **Methods**: A total of 8,187 clinical isolates were identified to the species level at each site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by each site using supplied broth microdilution panels and interpreted according to CLSI guidelines. Tigecycline breakpoint is defined as susceptible MICs ≤2 mcg/mL. **Results**: Various resistance patterns and phenotypes were detected among *Enterobacteriaceae* sampled in this study. As shown in the table below, tigecycline presented excellent inhibitory activity against all resistance phenotypes encountered.

	Tigecycline	
	%Susceptible	MIC_{90}
ESBL producing *E. coli* and *Klebsiella* isolates (n=354)	93.2	2
AmpC producing *Enterobacter* and *Serratia* isolates (n=303)	78.5	4
Fluoroquinolone resistant isolates (n=1643)	92.5	2
Aminoglycoside resistant isolates (n=38)	97.4	1
Isolates with reduced susceptibility to carbapenems (n=107)	90.7	2

Conclusion: Multi-drug resistance is often seen in health care acquired pathogens. The presented data suggest that tigecycline is highly potent against nosocomial or community pathogens regardless to the resistance patterns.

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 antibacterial 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 activity against aerobic and anaerobic Gram-positive and Gram-negative microorganisms [2, 3].

Most tetracycline-resistant bacteria with either tetracycline efflux pumps or ribosomal protective features are sensitive to tigecycline [3-5]. Tigecycline has shown to be highly effective against multi-resistant Acinetobacter spp., particularly A. baumannii, which are commonly associated with serious nosocomial infections [6]. Similar activity has been observed against *Enterobacteriaceae*, even extended-spectrum beta-lactamase and AmpC producing strains [7] and carbapenemase-producing Klebsiella [8].

This study was designed to better define the in vitro activity of tigecycline in a large diverse population of various resistant phenotypes of clinical *Enterobacteriaceae* collected from hospitals multi-center, multi-country global population.

MATERIALS & METHODS

- All isolates were derived from blood, respiratory tract, urine, skin, wound, body fluids and other defined sources. Only one isolate per patient was accepted into the study. Clinical isolates were collected and tested between January 2004 - July 2005 from 107 study centers in 25 countries. Isolates were identified to the species level and tested at each site by the participating laboratory.
- Organism collection, transport, confirmation of organism identification, as well as, development and management of a centralized database was coordinated by Laboratories International for Microbiology Studies (LIMS), a division of International Health Management Associates, Inc. located in Schaumburg, IL, USA.
- All organisms were deemed clinically significant by local participant criteria. Isolate inclusion was independent of medical history, antimicrobial use, age or gender. All sites identified each study isolate utilizing local laboratory site criteria.

Antimicrobial Susceptibility Testing

- Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [9]. Tigecycline was supplied by Wyeth Pharmaceuticals (Collegeville, PA, USA). All other agents were supplied by the panel manufacturer, MicroScan (Dade Behring Inc., Sacramento, CA, USA). The following antimicrobial agents were included on the panels with their dilution ranges (expressed in mcg/mL): amikacin (0.5-64); amoxicillin/clavulanic acid (0.12/0.06-32/16); ampicillin (0.5-32, Gram-negative panel) and (0.06-16, Gram-positive panel); cefepime (0.5-32); ceftriaxone (0.06-64); ceftazidime (8-32); imipenem (0.06-16); linezolid (0.5-8); levofloxacin (0.008-8); minocycline (0.5-16); tigecycline (0.008-16); penicillin (0.06-8); piperacillin/tazobactam (0.06/4-128/4) and vancomycin (0.12-32). MIC interpretive criteria followed published guidelines established by the Clinical and Laboratory Standards Institute [10] and recent US Food and Drug Administration packaging insert for tigecycline [11], where applicable.
- *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were screened for ESBL activity when MIC results for ceftriaxone were >1 mcg/ml using broth microdilution panels. ESBL activity was confirmed using the CLSI (2005) phenotypic confirmatory disk test (Oxoid, Ogdensburg, NY, USA) on Mueller-Hinton agar (Remel Inc., Lenexa, KS, USA) according to CLSI (2005) guidelines. ESBL presence was confirmed by testing the following antibiotic disks: cefotaxime (30 mcg), cefotaxime/clavulanic acid (30/10 mcg) and ceftazidime (30 mcg), ceftazidime/clavulanic acid (30/10 mcg). Antimicrobial disks were manufactured by Oxoid, Inc. (Ogdensburg, NY, USA). Mueller-Hinton agar used in testing was manufactured by Remel, Inc. (Lenexa, KS, USA). An organism was interpreted as containing an ESBL if there was an increase of >5 mm in the inhibition zone of the combination disk when compared to that of the cephalosporin alone.
- Quality controls (QC) were performed by each testing site on each day of testing using the corresponding ATCC control strains: *E. coli* ATCC 25922; *E. coli* ATCC 35218. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI (2005) guidelines [10].

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. 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.
4. 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.
5. Rupp, M.E. and P.D. Fey, Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*: considerations for diagnosis, prevention and drug treatment. Drugs, 2003. 63(4): p. 353-65.
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. 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.
8. Bratu, S., et al., Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, NY: molecular epidemiology and in vitro activity of polymyxin B and other agents. J Antimicrob Chemother, 2005. 56(1): p. 128-32.
9. CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Sixth Edition, in Document M7-A6. 2005: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
10. CLSI, Performance Standards for Antimicrobial Susceptibility Testing, in Document M100-S15. 2005: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
11. Tygacil, Product Insert. 2005: Wyeth Pharmaceuticals, Inc., Philadelphia, PA, USA.

ACKNOWLEDGEMENTS

We gratefully acknowledge the contributions of the investigators, laboratory personnel and all members of the Tigecycline Evaluation Study Trials program group. This study was sponsored by a grant from Wyeth Pharmaceuticals.

RESULTS

Table 1. In Vitro Activity of Tigecycline and ComparatorsThe results are listed in the following Tables.

Table 1. In Vitro Activity of Tigecycline and Comparators against 8,187 Enterobacteriaceae and Various Resistant Phenotypes from a Global Population

Organism	Drug	MIC (mcg/mL)			%Sus [a]
		MIC_{50}	MIC_{90}	Range	
Enterobacteriaceae (n=8187)	Tigecycline	0.5	1	≤0.008 - 8	96.5
	Amikacin	2	4	≤0.5 - 32	97.7
	AmoxClav	16	>32	≤0.12 - >32	47.5
	Ampicillin	>32	>32	≤0.5 - >32	14.3
	Cefepime	≤0.5	2	≤0.5 - >32	95.1
	Ceftazidime	≤8	32	≤8 - >32	85.5
	Ceftriaxone	0.12	32	≤0.06 - >64	87.1
	Imipenem	0.5	1	0.12 - >16	98.7
	Levofloxacin	0.06	8	≤0.008 - >8	86.5
	Minocycline	2	8	≤0.5 - >16	85.3
	Pip-tazo	1	32	≤0.06 - >128	89.9
ESBL producing *E. coli* and *Klebsiella* (n=354)	Tigecycline	0.5	2	0.06 - 8	93.2
	Amikacin	4	16	0.5 - 32	90.7
	AmoxClav	16	>32	2 - >32	27.4
	Ampicillin	>32	>32	4 - >32	0.3
	Cefepime	8	>32	0.5 - >32	50.8
	Ceftazidime	>32	>32	8 - >32	21.5
	Ceftriaxone	64	>64	0.06 - >64	20.3
	Imipenem	0.5	2	0.12 - >16	91
	Levofloxacin	8	>8	0.015 - >8	35.9
	Minocycline	4	>16	0.5 - >16	66.1
	Pip-tazo	8	>128	0.5 - >128	66.9
Aminoglycoside-resistant [b] *Enterobacteriaceae* (n=38)	Tigecycline	0.5	1	0.12 - 4	97.4
	Amikacin	>64	>64	64 - >64	0
	AmoxClav	32	>32	2 - >32	13.2
	Ampicillin	>32	>32	32 - >32	0
	Cefepime	32	>32	≤0.5 - >32	26.3
	Ceftazidime	≤8	>32	≤8 - >32	52.6
	Ceftriaxone	>64	>64	0.25 - >64	7.9
	Imipenem	0.5	1	0.25 - >16	97.4
	Levofloxacin	2	>8	0.03 - >8	60.5
	Minocycline	4	16	≤0.5 - >16	63.2
	Pip-tazo	4	>128	≤0.06 - >128	73.7
Enterobacteriaceae with Carbapenem reduced-Susceptibility [c] (n=107)	Tigecycline	1	2	0.06 - 8	90.7
	Amikacin	4	32	11689	80.4
	AmoxClav	>32	>32	2 - >32	8.4
	Ampicillin	>32	>32	1 - >32	3.7
	Cefepime	32	>32	<0.5 - >32	40.2
	Ceftazidime	>32	>32	<8 - >32	36.4
	Ceftriaxone	>64	>64	<0.06 - >64	32.7
	Imipenem	16	>16	8 - >16	0
	Levofloxacin	>8	>8	0.015 - >8	38.3
	Minocycline	4	16	<0.5 - >16	79.4
	Pip-tazo	>128	>128	1 - >128	35.5
Fluoroquinolone-resistant [d] *Enterobacteriaceae* (n=1643)	Tigecycline	0.25	2	0.06 - 8	92.5
	Amikacin	2	16	<0.5 - 32	95.1
	AmoxClav	16	>32	1 - >32	33.2
	Ampicillin	>32	>32	<0.5 - >32	4.1
	Cefepime	1	>32	<0.5 - >32	74.3
	Ceftazidime	≤8	>32	<8 - >32	57.4
	Ceftriaxone	2	>64	<0.06 - >64	56.7
	Imipenem	0.25	2	0.12 - >16	92.8
	Levofloxacin	>8	>8	8 - >8	0
	Minocycline	4	>16	<0.5 - >16	58.1
	Pip-tazo	4	>128	0.25 - >128	68.9
AmpC producing *Enterobacter* and *Serratia* [e] (n=303)	Tigecycline	1	4	0.06 - 8	78.5
	Amikacin	2	16	<0.5 - 32	97
	AmoxClav	>32	>32	8 - >32	1
	Ampicillin	>32	>32	32 - >32	0
	Cefepime	8	>32	<0.5 - >32	61.4
	Ceftazidime	>32	>32	32 - >32	0
	Ceftriaxone	>64	>64	64 - >64	0
	Imipenem	0.5	2	0.25 - >16	95.7
	Levofloxacin	1	>8	0.015 - >8	59.7
	Minocycline	4	>16	<0.5 - >16	55.8
	Pip-tazo	128	>128	0.5 - >128	16.5

[a]Susceptibilities and resistant phenotypes are according to interpretive criteria as defined by CLSI document M100-S15 (2005); tigecycline susceptible breakpoint as defined in the FDA package insert (Tygacil®, 2005) [11].
[b]Amikacin MIC >64 mcg/mL
[c]Imipenem MIC >8 mcg/mL
[d]Levofloxacin MIC >8 mcg/mL
[e]AmpC deducted by resistance to 3rd generation cephalosporins ceftazidime and ceftriaxone

CONCLUSIONS

- The in vitro activity of tigecycline was equivalent to imipenem, amikacin and cefepime against all study strains of Enterobacteriaceae without regard to resistance mechanisms.
- With in vitro MIC_{50} and MIC_{90} values of 0.5 and 1 mcg/mL, respectively, tigecycline inhibited the growth of 7897/8187 (96.5%) of the clinical strains of *Enterobacteriaceae.*
- Tigecycline was unaffected by the production of ESBL enzymes in *E. coli* and *Klebsiella* with MIC_{50} and MIC_{90} values of 0.5 and 2 mcg/mL, respectively, inhibiting the growth of 93.2% of these strains at its FDA susceptibility breakpoint of 2 mcg/mL. Only amikacin (90.7%) and imipenem (91.0%) had inhibition rates >90% against these ESBL producers at their respective CLSI breakpoints.
- Tigecycline and imipenem had equivalent activity against amikacin-resistant *Enterobacteriaceae* with MIC_{50} and MIC_{90} values of 0.5 and 1 mcg/mL and susceptible rates of 97.4%.
- Tigecycline was the only study drug to inhibit >90% of *Enterobacteriaceae* with reduced susceptibility to carbapenems. Tigecycline's MIC_{90} of 2 mcg/mL was 8- to 128-fold lower than comparators against these strains.
- Amikacin and imipenem were superior to all study drugs against AmpC producing *Enterobacter* and *Serratia* inhibiting >95% of all strains at their respective susceptibility breakpoints. Tigecycline inhibited 78.5% of AmpC producers at its FDA breakpoint of 2 mcg/mL.
- At a MIC_{90} value of 2 mcg/mL tigecycline activity was equivalent to amikacin and imipenem against fluoroquinolone-resistant *Enterobacteriaceae.*
- The in vitro activity of tigecycline in this study suggests that tigecycline is a potent antimicrobial agent against multiple drug resistant phenotypes of *Enterobacteriaceae* and may be a welcomed addition to the armamentarium of therapeutic agents against these challenging pathogens.