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Determining Tigecycline in vitro Activity against Multi-Drug Resistant Enterobacteriaceae for the T.E.S.T. Program - United States Data

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REVISED ABSTRACT

Background: Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the in vitro activity of tigecycline against multi-drug resistant *Enterobacteriaceae* for the 10 antimicrobials: amoxicillin-clavulanic acid, piperacillin-tazobactam, levofloxacin, ceftriaxone, cefepime, ampicillin, amikacin, minocycline, ceftazidime and imipenem. All isolates were collected from 77 clinical labs in the United States throughout 2004-2005. The objective of this study was to evaluate the activity of tigecycline against multi-resistant strains of *Enterobacteriaceae* commonly associated with serious clinical infections. **Methods:** A total of 5,760 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 (FDA, 2005). **Results:** As expected, different resistance patterns 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 %S	Tigecycline MIC_{90}
ESBL producing *E. coli* and *Klebsiella* isolates (n=166)	93.4	2
AmpC producing *Enterobacter* and *Serratia* isolates (n=106)	80.2	4
Fluoroquinolone (Levofloxacin) resistant isolates (n=630)	90.8	2
Aminoglycoside (Amikacin) resistant isolates (n=1)	100	2
Isolates with reduced susceptibility to imipenem (n=69)	89.9	4

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 (ESBL) 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 multi-center population within the United States.

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 77 study centers within the United States. 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; cefepime (0.5-32); ceftriaxone (0.06-64); ceftazidime (8-32); imipenem (0.06-16); levofloxacin (0.008-8); minocycline (0.5-16); tigecycline (0.008-16); and piperacillin/tazobactam (0.06/4-128/4). MIC interpretive criteria followed published guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [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].

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RESULTS

The results are listed in the following Tables.

Table 1. In Vitro Activity of Tigecycline and Comparators against 5,760 *Enterobacteriaceae* and Various Resistant Phenotypes from the United States.

Organism	Drug	MIC (mcg/mL) MIC_{50}	MIC_{90}	Range	%Sus[a]
Enterobacteriaceae (n=5760)	Tigecycline	0.5	1	≤0.008 - 8	96.5
	Amikacin	2	4	≤0.5 - >64	99.1
	AmoxClav	8	>32	≤0.12 - >32	50.2
	Ampicillin	>32	>32	≤0.5 - >32	15.9
	Cefepime	≤0.5	2	≤0.5 - >32	96.6
	Ceftazidime	≤8	32	≤8 - >32	87.4
	Ceftriaxone	≤0.06	8	≤0.06 - >64	90.1
	Imipenem	0.5	1	≤0.06 - >16	98.3
	Levofloxacin	0.06	8	≤0.008 - >8	87.2
	Minocycline	2	8	≤0.5 - >16	86.6
	PipTazo	1	16	≤0.06 - >128	91.1
ESBL producing *E. coli* and *Klebsiella* (n=166)	Tigecycline	1	2	0.06 - 8	93.4
	Amikacin	8	32	≤0.5 - 32	86.1
	AmoxClav	16	>32	2 - >32	27.7
	Ampicillin	>32	>32	16 - >32	0
	Cefepime	8	>32	≤0.5 - >32	54.2
	Ceftazidime	>32	>32	≤8 - >32	9.6
	Ceftriaxone	64	>64	≤0.06 - >64	25.3
	Imipenem	0.5	8	0.25 - >16	78.9
	Levofloxacin	>8	>8	0.03 - >8	25.3
	Minocycline	4	>16	≤0.5 - >16	69.3
	PipTazo	16	>128	0.5 - >128	51.2
Aminoglycoside-resistant[b] *Enterobacteriaceae* (n=1)[f]	Tigecycline	-	-	2	100
	Amikacin	-	-	>64	0
	AmoxClav	-	-	>32	0
	Ampicillin	-	-	>32	0
	Cefepime	-	-	>32	0
	Ceftazidime	-	-	>32	0
	Ceftriaxone	-	-	>64	0
	Imipenem	-	-	0.5	100
	Levofloxacin	-	-	2	100
	Minocycline	-	-	16	0
	PipTazo	-	-	64	0
Enterobacteriaceae with Carbapenem reduced-Susceptibility[c] (n=96)	Tigecycline	1	4	0.12 - 8	89.6
	Amikacin	4	32	1 - 32	77.1
	AmoxClav	>32	>32	2 - >32	9.4
	Ampicillin	>32	>32	2 - >32	5.2
	Cefepime	32	>32	≤0.5 - >32	37.5
	Ceftazidime	>32	>32	≤8 - >32	34.4
	Ceftriaxone	>64	>64	≤0.06 - >64	30.2
	Imipenem	16	>16	8 - >16	0
	Levofloxacin	>8	>8	0.015 - >8	34.4
	Minocycline	4	16	≤0.5 - >16	80.2
	PipTazo	>128	>128	1 - >128	35.4
Fluoroquinolone-resistant[d] *Enterobacteriaceae* (n=630)	Tigecycline	0.25	2	0.06 - 8	90.8
	Amikacin	2	16	≤0.5 - 32	93.7
	AmoxClav	16	>32	1 - >32	33
	Ampicillin	>32	>32	≤0.5 - >32	4.6
	Cefepime	≤0.5	>32	≤0.5 - >32	78.6
	Ceftazidime	≤8	>32	≤8 - >32	55.1
	Ceftriaxone	1	>64	≤0.06 - >64	60.5
	Imipenem	0.5	4	0.25 - >16	90
	Levofloxacin	>8	>8	8 - >8	0
	Minocycline	4	>16	≤0.5 - >16	60.2
	PipTazo	4	>128	0. 5 - >128	63.8
AmpC producing *Enterobacter* and *Serratia*[e] (n=106)	Tigecycline	0.5	4	0..25 - 8	80.2
	Amikacin	2	8	1 - 32	98.1
	AmoxClav	>32	>32	16 - >32	0
	Ampicillin	>32	>32	>32 - >32	0
	Cefepime	4	8	≤0.5 - 8	100
	Ceftazidime	>32	>32	32 - >32	0
	Ceftriaxone	64	>64	64 - >64	0
	Imipenem	0.5	1	0.25 - >16	99.1
	Levofloxacin	0.25	>8	0.015 - >8	64.2
	Minocycline	4	>16	≤0.5 - >16	60.4
	PipTazo	64	>128	0.5 - >128	10.4

[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 3[rd] generation cephalosporins ceftazidime and ceftriaxone and susceptibility to cefepime.
[f]$MIC_{50/90}$ not calculated for groups with n <10.

CONCLUSIONS

- Tigecycline inhibited 97% of all *Enterobacteriaceae* tested in vitro at its defined susceptibility breakpoint MIC of 2 mcg/mL. The percentage of strains susceptible to tigecycline was comparable to that seen with imipenem (98%), cefepime (97%) and amikacin (99%).
- Tigecycline's MIC_{90} of 1 mcg/mL was 8 to 64 fold better than the beta-lactams, beta-lactam/beta-lactamase inhibitor combinations and levofloxacin against all *Enterobacteriaceae* tested.
- Tigecycline demonstrated potent in vitro activity against both ESBL producing *E. coli*, *K. pneumoniae* and *K. oxytoca*.
- Tigecycline demonstrated potent in vitro activity against both AmpC producing *Enterobacter* and *S. marcescens*.
- Tigecycline demonstrated potent in vitro activity against *Enterobacteriaceae* with reduced susceptibility to imipenem.
- The one study strain resistant to amikacin was susceptible to tigecycline.
- The in vitro activity of tigecycline in this study suggests that tigecycline is a potent antimicrobial agent in the treatment of gram-negative infections caused by selected strains of *Enterobacteriaceae* with or without multi-drug resistance.