

REVISED ABSTRACT

Background: Tigecycline 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 compared to most commonly prescribed broad spectrum antimicrobials against gram negative and gram positive species collected during 2004 to 2006. **Methods:** A total of 1,446 clinical isolates from five Eastern European testing sites were identified to the species level. Minimum Inhibitory Concentration (MICs) were determined by each site using supplied broth microdilution panels and interpreted according to EUCAST guidelines where available; CLSI breakpoints, where available, were used where no EUCAST breakpoints exist. **Results:** Selective results are presented as follows:

<i>E. coli</i> and <i>Klebsiella</i> spp.				<i>Acinetobacter</i> spp.			
In patients (n=300)		Out patients (n=31)		In patients (n=106)		Out patients (n=5)	
%	MIC ₉₀	%	MIC ₉₀	%	MIC ₉₀	%	MIC ₉₀
Tigecycline	93.7	1	93.5	1	na	1	na
Amikacin	87	16	90.3	4	60.4	>64	20
Cefepime	70.7	32	77.4	8	42	>32	0
Ceftazidime	0	>32	0	16	35.8	>32	0
Imipenem	97.6	0.5	100	0.5	75.5	16	>16
Levofloxacin	72.7	8	64.5	>8	39.6	>8	0
Minocycline	93.7	8	87.1	8	98.1	1	100
PipTazo	81.7	128	93.5	32	38.7	>128	0

<i>S. aureus</i>				<i>Enterococcus</i> spp.			
In patients (n=127)		Out patients (n=9)		In patients (n=91)		Out patients (n=11)	
%	MIC ₉₀	%	MIC ₉₀	%	MIC ₉₀	%	MIC ₉₀
Tigecycline	100	0.25	100	0.25	100	0.12	100
Levofloxacin	70.9	4	60	4	72.2	>32	87.5
Linezolid	100	2	100	4	100	2	100
Minocycline	98.4	4	93.3	4	59.3	8	62.5
Vancomycin	100	1	100	1	98.1	2	100

Conclusion: Tigecycline's in vitro activity was comparable to or greater than most commonly prescribed broad spectrum antimicrobials without any demonstrable change in activity between in- and out-patient bacterial study strains. Tigecycline's activity against *E. coli*, *Klebsiella* spp. and *Acinetobacter* spp. was comparable to imipenem. Against gram positive organisms, tigecycline's activity was similar to linezolid and vancomycin.

INTRODUCTION

Tigecycline (formerly GAR-936) is a member of a new class of antimicrobial agents, the glycolcyclines. This synthetic analogue of the tetracyclines exhibits significant antibacterial activity that is both bacteriostatic and, in certain instances, bactericidal with killing activity that is as much as fourfold better than vancomycin and daptomycin [1, 2]. The development of tigecycline is important in that tigecycline and other glycolcyclines are active against bacterial strains carrying either or both of the two major forms of tetracycline resistance: efflux and ribosomal protection. Certain substituents at the 9-position of the tetracycline molecule restored activity against bacteria harboring genes encoding either or both efflux and ribosomal protection. A single chemical modification of tigecycline overcomes the two molecularly distinct forms of resistance while maintaining activity against susceptible gram-positive, gram-negative, aerobic, and anaerobic bacteria [3]. Furthermore, resistance to tigecycline is difficult to produce even in the laboratory.

Previous studies have demonstrated excellent in vitro activity for tigecycline against clinical and laboratory strains of gram-positive and -negative bacteria with minimum inhibitory concentrations for the 90th percentile inhibited at or below 2 mcg/ml, including difficult-to- treat methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* [4-6]. This study was undertaken to document the in vitro activity of tigecycline against significant numbers of clinical isolates collected in five Eastern European countries from in-patient and out-patient populations. This study is part of the larger ongoing global Tigecycline Evaluation and Surveillance Trials (T.E.S.T.) program.

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 2004 to 2006 from 8 study centers in the Czech Republic, Greece, Hungary, Latvia and Poland. 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.
- Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [7]. 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); ceftazidime (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 EUCAST [9] and CLSI where none currently exist with EUCAST [8].
- Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were screened for ESBL activity when MIC results for ceftazidime were >1mcg/ml using broth microdilution panels. ESBL activity was confirmed using the CLSI (2006) phenotypic confirmatory disk test (Oxoid, Ogdensburg, NY, USA) on Mueller-Hinton agar (Remel Inc., Lenexa, KS, USA) according to CLSI (2006) guidelines. ESBL presence was confirmed by testing the following antibiotic disks: cefotaxime (30-mcg), cefotaxime/clavulanic acid (30/10-mcg), ceftazidime (30-mcg), and 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: *Enterococcus faecalis* ATCC 29212; *Escherichia coli* ATCC 25922; *Klebsiella pneumoniae* ATCC 700603 (positive ESBL control); *Haemophilus influenzae* ATCC 49247; *Haemophilus influenzae* ATCC 49766; *Staphylococcus aureus* ATCC 29213; *Streptococcus pneumoniae* ATCC 49619; and *Pseudomonas aeruginosa* ATCC 27853. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI (2006) guidelines [8].

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RESULTS

The results are listed in the following Tables.

Table 1. In vitro activity of tigecycline and comparative antimicrobial agents against gram-negative rods isolated from in-patients and out-patients - Eastern European results.

Organism (N)	Drug	In-Patients			Out-Patients		
		MIC ₉₀ ^a	MIC ₅₀	%Sus ^b	MIC ₉₀	MIC ₅₀	%Sus
<i>E. coli</i> , <i>K. oxytoca</i> , <i>K. pneumoniae</i> In-Patient (n=300) Out-Patient (n=31)	Tigecycline	0.25	1	93.7	0.25	1	93.5
	Amikacin	2	16	87	2	4	90.3
	AmoxClav	8	32	61	8	16	90.3
	Ampicillin	>32	>32	18.3	>32	>32	77.4
	Cefepime	<0.5	32	70.7	<0.5	8	77.4
	Ceftazidime	<8	>32	0	<8	16	0
	Ceftriaxone	<0.06	>64	68.3	<0.06	64	74.2
	Imipenem	0.25	0.5	97.6	0.5	0.5	100
	Levofloxacin	0.03	8	72.7	0.06	>8	64.5
	Minocycline	1	8	80.7	2	8	87.1
PipTazo	1	128	81.7	1	32	93.5	
All ESBLs ^c In-Patient (n=60) Out-Patient (n=4) ^d	Tigecycline	0.5	4	81.7	-	-	75
	Amikacin	8	64	58.3	-	-	50
	AmoxClav	16	>32	23.3	-	-	25
	Ampicillin	>32	>32	0	-	-	0
	Cefepime	8	>32	6.7	-	-	0
	Ceftazidime	>32	>32	0	-	-	0
	Ceftriaxone	64	>64	1.7	-	-	0
	Imipenem	0.25	0.5	98.3	-	-	100
	Levofloxacin	4	>8	45	-	-	25
	Minocycline	4	>16	60	-	-	75
PipTazo	8	>128	63.3	-	-	50	
<i>E. aerogenes</i> In-Patient (n=22) Out-Patient (n=0)	Tigecycline	0.5	2	86.4	-	-	-
	Amikacin	2	8	90.9	-	-	-
	AmoxClav	>32	>32	0	-	-	-
	Ampicillin	>32	>32	0	-	-	-
	Cefepime	<0.5	16	50	-	-	-
	Ceftazidime	32	>32	0	-	-	-
	Ceftriaxone	4	64	36.4	-	-	-
	Imipenem	0.5	1	90.9	-	-	-
	Levofloxacin	0.06	>8	68.2	-	-	-
	Minocycline	2	16	81.8	-	-	-
PipTazo	32	128	45.5	-	-	-	
<i>E. cloacae</i> In-Patient (n=131) Out-Patient (n=9) ^d	Tigecycline	0.5	2	87	-	-	100
	Amikacin	1	4	92.4	-	-	88.9
	AmoxClav	>32	>32	0	-	-	0
	Ampicillin	>32	>32	0	-	-	0
	Cefepime	<0.5	8	73.3	-	-	77.8
	Ceftazidime	<8	>32	0	-	-	0
	Ceftriaxone	0.25	>64	58.8	-	-	44.4
	Imipenem	0.5	0.5	100	-	-	100
	Levofloxacin	0.06	1	90.1	-	-	77.8
	Minocycline	2	8	83.2	-	-	77.8
PipTazo	2	64	74	-	-	77.8	
<i>E. coli</i> In-Patient (n=161) Out-Patient (n=19)	Tigecycline	0.12	0.25	100	0.12	0.25	100
	Amikacin	2	16	88.8	2	4	100
	AmoxClav	8	32	67.7	8	16	84.2
	Ampicillin	>32	>32	34.2	>32	>32	42.1
	Cefepime	<0.5	8	80.7	<0.5	8	84.2
	Ceftazidime	<8	16	0	<8	78	0
	Ceftriaxone	<0.06	>64	79.5	<0.06	64	84.2
	Imipenem	0.25	0.5	99.4	0.25	0.5	100
	Levofloxacin	0.03	8	77.6	0.03	>8	68.4
	Minocycline	1	8	83.2	1	8	73.7
PipTazo	1	8	92.5	1	2	100	
<i>E. coli</i> , ESBL ^c In-Patient (n=24) Out-Patient (n=2) ^d	Tigecycline	0.12	0.25	100	-	-	100
	Amikacin	8	64	50	-	-	100
	AmoxClav	16	32	29.2	-	-	50
	Ampicillin	>32	>32	0	-	-	0
	Cefepime	8	>32	4.2	-	-	0
	Ceftazidime	32	>32	0	-	-	0
	Ceftriaxone	64	>64	4.2	-	-	0
	Imipenem	0.25	2	95.8	-	-	100
	Levofloxacin	0.25	>8	54.2	-	-	50
	Minocycline	4	16	66.7	-	-	100
PipTazo	8	64	79.2	-	-	100	
<i>K. oxytoca</i> In-Patient (n=22) Out-Patient (n=0)	Tigecycline	0.25	1	95.5	-	-	-
	Amikacin	1	2	95.5	-	-	-
	AmoxClav	2	16	81.8	-	-	-
	Ampicillin	>32	>32	0	-	-	-
	Cefepime	<0.5	1	90.9	-	-	-
	Ceftazidime	-	-	0	-	-	-
	Ceftriaxone	-	-	100	-	-	-
	Imipenem	-	-	0	-	-	-
	Levofloxacin	-	-	0	-	-	-
	Minocycline	-	-	100	-	-	-
PipTazo	-	-	100	-	-	-	
<i>K. pneumoniae</i> In-Patient (n=117) Out-Patient (n=12) ^d	Tigecycline	0.5	2	84.6	-	-	83.3
	Amikacin	2	16	82.9	-	-	75
	AmoxClav	16	>32	47.9	-	-	66.7
	Ampicillin	>32	>32	0	-	-	0
	Cefepime	<0.5	>32	53	-	-	66.7
	Ceftazidime	<8	>32	0	-	-	0
	Ceftriaxone	4	>64	49.6	-	-	58.3
	Imipenem	0.25	0.5	94.7	-	-	100
	Levofloxacin	0.5	>8	63.2	-	-	58.3
	Minocycline	2	>16	76.1	-	-	83.3
PipTazo	4	>128	65	-	-	66.7	
<i>K. pneumoniae</i> , ESBL ^c In-Patient (n=35) Out-Patient (n=2) ^d	Tigecycline	0.5	4	68.6	-	-	50
	Amikacin	8	16	65.7	-	-	0
	AmoxClav	16	>32	20	-	-	0
	Ampicillin	>32	>32	0	-	-	0
	Cefepime	8	>32	8.6	-	-	0
	Ceftazidime	>32	>32	0	-	-	0
	Ceftriaxone	32	>64	0	-	-	0
	Imipenem	0.25	0.5	100	-	-	100
	Levofloxacin	4	>8	40	-	-	0
	Minocycline	4	>16	54.3	-	-	50
PipTazo	16	>128	51.4	-	-	0	
<i>S. marcescens</i> In-Patient (n=54) Out-Patient (n=4) ^d	Tigecycline	1	2	79.6	-	-	100
	Amikacin	2	8	90.7	-	-	100
	AmoxClav	>32	>32	1.9	-	-	0
	Ampicillin	>32	>32	0	-	-	0
	Cefepime	<0.5	1	90.7	-	-	100