

Tigecycline Evaluation Surveillance Trial (T.E.S.T.) Program - In Vitro Antibacterial Activity Against Selected Species of *Enterobacteriaceae* in the United States

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S. Bouchillon¹, T. Stevens¹, B. Johnson¹, J. Johnson¹, D. Hoban¹, M. Hackel¹, M. Person¹, M. Dowzicky²

¹International Health Management Associates, Schaumburg, IL, USA

²Wyeth 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 a potent expanded broad spectrum activity against most species of Enterobacteriaceae as well as gram-positive, atypicals and anaerobes. The T.E.S.T. program determined the in vitro activity of tigecycline compared to amikacin, ampicillin, amoxicillin/clavulanic acid, imipenem, cefepime, ceftazidime, ceftriaxone, levofloxacin, minocycline and piperacillin/tazobactam against isolates of *Enterobacteriaceae* collected from 44 hospitals across the United States. **Methods:** A total of 5,012 clinical isolates, collected in 2004, were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using broth microdilution panels from Dade Behring and interpreted according to CLSI guidelines. **Results:** The efficacy of all broad spectrum antimicrobial agents still remain highly active against *Enterobacteriaceae* in the United States. The susceptibility rates for amikacin, cefepime, ceftazidime, ceftriaxone, imipenem, levofloxacin, minocycline and piperacillin/tazobactam are 98.9%, 96.8%, 89.4%, 91.8%, 98.2%, 86.1%, 86% and 92.3%, respectively. Tigecycline's activity was similar to imipenem presenting a MIC₅₀/MIC₉₀ of 0.25/1 mcg/ml against all strains of *Enterobacteriaceae*. The frequency of ESBL production among *K. pneumoniae*, *K. oxytoca* and *E. coli* was found to be 8.4%, 3.7%, and 1.6%, respectively. Tigecycline successfully inhibited >98% of all ESBL producers at a MIC of 4 mcg/ml. Unusual resistance to imipenem was noticed in 1.8% of isolates. While still under more detailed analysis, preliminary data have shown that tigecycline presented a MIC₅₀/MIC₉₀ of 1/2 mcg/ml against these imipenem-resistant isolates. **Conclusion:** Most of broad spectrum antimicrobial agents still remain active against *Enterobacteriaceae* from the US. Tigecycline's activity was comparable to the activities of broad spectrum antimicrobials and with greater activity against most ESBL and AmpC producing isolates. Tigecycline also showed in vitro activity against isolates that were intermediate or resistant to imipenem. The presented data suggest that tigecycline may be an effective and reliable therapeutic option against both susceptible strains of *Enterobacteriaceae* and resistant strains regardless of degree or type of resistance.

INTRODUCTION

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 was developed to provide activity against tetracycline and 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, 7-11]. This broad spectrum activity has been demonstrated against gram-negative pathogens, even extended-spectrum β-lactamase producing *E. coli* and *Klebsiella pneumoniae* [10, 12]. This study was designed to better define tigecycline activity in a large diverse population of clinical isolates.

This study compared the activity of tigecycline with other agents against *Enterobacteriaceae* including *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Serratia marcescens* from hospitals across United States.

MATERIALS & METHODS

- All isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, fluids and few other defined sources. Only one isolate per patient was accepted.
- Clinical isolates were collected tested between January 2004 - December 2004 from 44 study centers across United States.
- Escherichia coli* and *Klebsiella pneumoniae* were screened and confirmed for ESBL activity according to CLSI guidelines (Table 2A, M100-S14) [13].
- ESBL activity 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). Antibiotic disks were manufactured by Oxoid Inc. Ogdensburg, New York. Mueller-Hinton agar used in testing was manufactured by Remel Inc. Lenexa, Kansas.

- An organism is interpreted as producing an ESBL if there is an increase of ≥ 5mm in the inhibition zone of the combination disc when compared to that of the cephalosporin alone: cefotaxime/clavulanic acid - cefotaxime ≥ 5 mm or ceftazidime/clavulanic acid - ceftazidime ≥ 5 mm.
- Antimicrobial agents tested with concentrations (expressed in mcg/ml) were: amoxicillin/clavulanic acid (0.12-32); piperacillin/tazobactam (0.06-128); levofloxacin (0.008-8); ceftriaxone (0.06-64); cefepime (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 CLSI where applicable [15]. 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 antibiotic disks followed manufacturers guidelines (Oxoid) using the following ATCC strains: *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922.
- 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).

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RESULTS

Table 1. In vitro activity of tigecycline and comparative agents against 5,012 strains of *Enterobacteriaceae*.

Organism Name	Drug	MICs (mcg/mL)		
		MIC ₅₀	MIC ₉₀	Range
<i>Enterobacteriaceae</i> (n=5,012)	Tigecycline	0.25	1	0.03-8
	Amikacin	2	4	<0.5->64
	Amox-Clav	8	>32	0.12->32
	Cefepime	≤0.5	1	0.5->32
	Ceftazidime	≤8	16	<8->32
	Ceftriaxone	≤0.06	8	<0.06->64
	Imipenem	0.5	1	0.12->16
Levofloxacin	0.06	8	<0.008->8	
Minocycline	2	8	<0.5->16	
Pip-Tazo	1	16	<0.06->128	

Table 2. In vitro activity of tigecycline and comparators against 5,012 stains of *Enterobacteriaceae* showing frequency distribution and cumulative percent inhibited (%) at each MIC (mcg/ml).

MIC	≤0.008	0.015	0.03	≥0.06	0.06	≤0.12	0.12	0.25	≤0.5	0.5	1	2	4	≤8	8	≥8	16	>16	32	>32	64	>64	128	>128	
Tigecycline	9	279	1197	1243	1430	518	188	118	30																
Amikacin	0.2	5.7	29.6	54.4	83	93.3	97	99.4	100																
Amox-Clav					74	1428	2473	668	220	95	45	7	1												
Cefepime					1.5	30	79.3	92.7	97	98.9	99.8	100	100												
Ceftazidime					0	31	216	1379	823	638	366	341	1217												
Ceftriaxone						0.5	4.9	32.5	48.9	61.6	68.9	75.7	100												
Imipenem						0.2	48.4	85.1	95.4	97.6	98.2	98.8	99.2	100											
Levofloxacin	25	527	1680	1133	364	214	183	116	73	76	205	416													
Minocycline	0.5	11	44.5	67.1	74.4	78.7	82.3	84.6	86.1	87.6	89.7	91.7	100												
Pip-Tazo							791	6	1375	1318	822	353	204	143											
							15.8	15.9	43.3	69.6	86	93.1	97.1	100											
							696	1602	1275	445	183	121	64												
							0.6	2	15.9	51.9	77.3	86.2	89.8	92.3	93.5	94.9	96.3	100							

Table 3. In vitro activity of tigecycline and comparative agents against major representatives of *Enterobacteriaceae*.

Organism Name	Drug ^a	MICs (mcg/mL)				
		%SUS	%INT	%RES	MIC ₅₀	MIC ₉₀
<i>Enterobacter aerogenes</i> (n=258)	Tigecycline	95.7	3.5	0.8	0.5	1
	Amikacin	98.4	1.6	0	2	4
	Amox-Clav	7	3.5	89.5	>32	>32
	Cefepime	98.4	0.8	0.8	≤0.5	1
	Ceftazidime	84.5	6.2	9.3	≤8	16
	Ceftriaxone	93.8	5.4	0.8	0.12	8
	Imipenem	98.4	0	1.6	1	2
	Levofloxacin	95	2.7	2.3	0.06	0.5
	Minocycline	89.9	5	5.1	2	8
	Pip-Tazo	89.5	6.6	3.9	2	32
<i>Enterobacter cloacae</i> (n=629)	Tigecycline	93.5	4.4	2.1	0.5	2
	Amikacin	99.2	0.5	0.3	2	2
	Amox-Clav	3.5	2.1	94.4	>32	>32
	Cefepime	95.5	1.4	3.1	≤0.5	4
	Ceftazidime	77.7	3.3	19	≤8	>32
	Ceftriaxone	80	8.7	11.3	0.25	64
	Imipenem	98.7	0.2	1.1	0.5	1
	Levofloxacin	92.1	1.7	6.2	0.06	2
	Minocycline	85.2	6.2	8.6	2	8
	Pip-Tazo	84.1	8.3	7.6	2	64

Organism Name	Drug	%SUS	%INT	%RES	MIC ₅₀	MIC ₉₀
<i>Escherichia coli</i> (n=951)	Tigecycline	100	0	0	0.12	0.25
	Amikacin	99.5	0.4	0.1	2	4
	Amox-Clav	77.1	12.6	10.3	4	32
	Cefepime	98.5	0.3	1.2	≤0.5	≤0.5
	Ceftazidime	95.2	1.2	3.6	≤8	≤8
	Ceftriaxone	96.3	1.3	2.4	≤0.06	0.12
	Imipenem	99.7	0	0.3	0.25	0.5
	Levofloxacin	77.9	1.6	20.5	0.03	>8
	Minocycline	85.9	8.2	5.9	1	8
	Pip-Tazo	96	1.7	2.3	1	4
	<i>Klebsiella pneumoniae</i> (n=807)	Tigecycline	95.2	3.9	0.9	0.5
Amikacin		97.9	1.9	0.2	2	4
Amox-Clav		84.9	5.7	9.4	2	16
Cefepime		94.7	0.7	4.6	≤0.5	2
Ceftazidime		87.1	1.4	11.5	≤8	>32
Ceftriaxone		90.7	4	5.3	≤0.06	8
Imipenem		96.2	1.6	2.2	0.25	1
Levofloxacin		88.2	1.1	10.7	0.06	8
Minocycline		84.1	6.6	9.3	2	8
Pip-Tazo		91	1.2	7.8	2	16
<i>Klebsiella oxytoca</i> (n=106)		Tigecycline	99.1	0.9	0	0.25
	Amikacin	100	0	0	2	4
	Amox-Clav	86.8	2.8	10.4	2	32
	Cefepime	100	0	0	≤0.5	1
	Ceftazidime	94.3	0	5.7	≤8	≤8
	Ceftriaxone	95.3	3.8	0.9	≤0.06	2
	Imipenem	99.1	0	0.9	0.25	0.5
	Levofloxacin	97.2	2.8	0	0.03	0.5
	Minocycline	92.5	6.6	0.9	1	4
	Pip-Tazo	91.5	0.9	7.6	1	16
	<i>S. marcescens</i> (n=348)	Tigecycline	96	3.7	0.3	1
Amikacin		100	0	0	2	4
Amox-Clav		2.3	1.4	96.3	>32	>32
Cefepime		96.6	1.7	1.7	≤0.5	1
Ceftazidime		91.4	3.1	5.5	≤8	≤8
Ceftriaxone		92	3.7	4.3	0.25	8
Imipenem		98.3	0.6	1.1	1	2
Levofloxacin		96.3	0.8	2.9	0.12	1
Minocycline		89.9	6	4.1	4	8
Pip-Tazo		95.1	2.6	2.3	1	8

^aBreakpoints as defined by NCCLS (M100-S14), 2004. Tigecycline breakpoints defined as: susceptible ≤ 2; intermediate = 4; and resistant ≤ 8

Table 4. In vitro activity of tigecycline and comparative agents against extended-spectrum beta-lactamase producing *Enterobacteriaceae*.

Organism Name	Drug ^a	MICs (mcg/mL)				
		%SUS	%INT	%RES	MIC ₅₀	MIC ₉₀
<i>Escherichia coli</i> , ESBL (n=15)	Tigecycline	100	0	0	0.12	0.25
	Amikacin	100	0	0	4	16
	Amox/Clav	6.7	46.6	46.7	16	32