

# Tigecycline Evaluation Surveillance Trial (T.E.S.T.) - In Vitro Antibacterial Activity Against Gram-positive and Gram-negative Pathogens in Asia and Pacific Rim

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## 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. The T.E.S.T. program determined the in vitro activity of tigecycline compared to amikacin, ampicillin, imipenem, cefepime, ceftazidime, ceftazidime/ceftriaxone, levofloxacin, minocycline and piperacillin/tazobactam against gram negative rods in addition to linezolid, penicillin and vancomycin for the gram positive species. Isolates were collected from hospitals located in Asia throughout 2004. **Methods:** A total of 1,005 clinical isolates 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 supplied broth microdilution panels and interpreted according to NCCLS guidelines. **Results:** Tigecycline's activity was similar to imipenem against *Enterobacteriaceae* with MIC<sub>50</sub>/MIC<sub>90</sub> of 0.5/1 mcg/ml. Resistance to third generation cephalosporin was found in 27.6% of *E. coli* and 31.5% of *K. pneumoniae* consistent with ESBL phenotype. Tigecycline inhibited majority of ESBL with MICs equal or less than 1 mcg/ml. Although similar to other classes of broad spectrum antimicrobial agents against glucose non-fermenters, tigecycline was especially active against *A. baumannii* presenting the lowest MIC<sub>90</sub> of 1 mcg/ml. Resistance to methicillin was detected in 39.4% of *S. aureus*. Tigecycline successfully inhibited *S. aureus* with MIC<sub>50</sub> of 0.25 mcg/mL regardless of sensitivity or resistance to methicillin. Tigecycline also showed the lowest MIC<sub>50</sub> against *Enterococcus* spp. of 0.5 mcg/ml. Against the fastidious pathogens, tigecycline inhibited the growth all *S. pneumoniae* and *H. influenzae* at MICs < 1 mcg/mL and was unaffected, respectively, by penicillin susceptibility phenotype or the production of beta-lactamase. **Conclusion:** Tigecycline's in vitro activity was comparable to or greater than most commonly prescribed antimicrobials. The presented data suggest that tigecycline may be an effective and reliable therapeutic option against both aerobic gram-positive and aerobic gram-negative bacteria, including multi-drug 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 multi-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 select for in the laboratory [5, 6] with a selection frequency observed at less than 10<sup>-9</sup> [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]. Tigecycline has shown to be highly effective against multi-resistant *Acinetobacter* spp., particularly *A. baumannii* that are commonly associated with serious nosocomial infections. Tigecycline is active against *Enterobacteriaceae*, including ESBL producing *E. coli*, *K. pneumoniae* and AmpC producing isolates [10, 12]. Against gram positive organisms, tigecycline MIC<sub>50</sub> values of <0.5 mcg/ml have been demonstrated against methicillin-resistant *Staphylococcus aureus* (MRSA) [2, 4-6]. Tigecycline has also shown potent activity in animal models infected with selected strains of multi-drug resistant *Enterococcus faecium* and *Enterococcus faecalis* [4, 5] with diverse genotypes vanA, -B and -C [6].

This study was designed to better define tigecycline's activity in a large diverse population of clinical isolates collected from hospitals in Australia, China, Singapore, India, Pakistan, and The Philippines.

## MATERIALS & METHODS

- All isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, fluids and other defined sources. Only one isolate per patient was accepted.
- Clinical isolates were collected and tested between January 2004 - December 2004 from 6 study centers in 6 countries.
- 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); Ampicillin (0.5-32); Amikacin (0.5-64); Minocycline (0.5-16); Ceftazidime (8-32); Tigecycline (0.008-16); Imipenem (0.06-16); Penicillin (0.06-8); Ampicillin (0.08-16); Linezolid (0.5-8), and Vancomycin (0.12-32). MIC interpretive criteria followed published guidelines established

by the NCCLS where applicable [15]. Tigecycline tentative breakpoints (in units of mcg/mL) are defined as susceptible ≤ 2; intermediate = 4; and resistant > 8.

- ESBL activity was confirmed by testing the following antibiotic disks: cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), and ceftazidime (30 µg), ceftazidime/clavulanic acid (30/10 µg). Antibiotic disks were manufactured by Oxoid Inc. Ogdenburg, 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.
- Isolates were identified to the genus and species level at each site by the local laboratory. The MICs of each isolate were determined 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), Schaumburg, IL USA.

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## RESULTS

Table 1. In Vitro Activity of Tigecycline and Comparative Agents against *Enterobacteriaceae*

Organism Name <sup>a</sup>	Drug <sup>b</sup>	MIC (mcg/mL)				
		%SUS	%INT	%RES	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Enterobacteriaceae</i> (n=404)	Tigecycline	98.8	0.7	0.5	0.5	1
	Amikacin	97.3	0	2.7	2	4
	Amox-Clav	41.3	14.4	44.3	16	>32
	Ampicillin	12.9	4.7	82.4	>32	>32
	Cefepime	91.1	3.2	5.7	≤0.5	8
	Ceftazidime	90	5.9	14.1	<8	32
	Ceftriaxone	75.2	6.7	18.1	0.12	>64
	Imipenem	99.5	0.2	0.2	0.5	1
	Levofloxacin	80.4	2.5	17.1	0.06	>8
	Minocycline	81.4	10.2	8.4	2	8
	Pip-Tazo	91.1	6.4	2.5	2	16
<i>E. coli</i> (n=123)	Tigecycline	100	0	0	0.12	0.25
	Amikacin	96.7	0	3.3	2	8
	Amox-Clav	67.5	26.8	5.7	8	16
	Ampicillin	29.5	0	70.5	>32	>32
	Cefepime	87	6.5	6.5	≤0.5	16
	Ceftazidime	87.8	5.7	6.5	>8	16
	Ceftriaxone	72.4	6.5	21.1	≤0.06	>64
	Imipenem	100	0	0	0.25	0.5
	Levofloxacin	57.7	4.1	38.2	0.5	>8
	Minocycline	66.7	22.8	10.6	2	16
	Pip-Tazo	98.4	0.8	0.8	1	4
<i>K. pneumoniae</i> (n=108)	Tigecycline	97.2	2.8	0	0.5	1
	Amikacin	95.4	0	4.6	1	8
	Amox-Clav	60.2	18.5	21.3	4	>32
	Ampicillin	5.6	6.5	88	>32	>32
	Cefepime	88.9	4.6	6.5	≤0.5	16
	Ceftazidime	75	6.5	18.5	>8	>32
	Ceftriaxone	68.5	9.3	22.2	0.12	>64
	Imipenem	100	0	0	0.5	1
	Levofloxacin	84.3	0.9	14.8	0.06	8
	Minocycline	84.1	5.6	10.3	2	16
	Pip-Tazo	92.6	3.7	3.7	2	8
<i>K. oxytoca</i> (n=16)	Tigecycline	100	0	0	0.25	1
	Amikacin	100	0	2	4	4
	Amox-Clav	68.8	0	31.3	4	>32
	Ampicillin	0	12.5	87.5	>32	>32
	Cefepime	100	0	0	≤0.5	≤0.5
	Ceftazidime	100	0	0	>8	>8
	Ceftriaxone	93.8	6.3	0	≤0.06	4
	Imipenem	100	0	0	0.5	0.5
	Levofloxacin	87.5	0	12.5	0.06	8
	Minocycline	100	0	0	1	2
	Pip-Tazo	81.3	0	18.8	2	>128
All ESBL producers	Tigecycline	98	2	0	0.5	1
	Amikacin	90.2	0	9.8	2	8
	Amox-Clav	25.5	58.8	15.7	16	32
	Ampicillin	2	0	98	>32	>32
	Cefepime	60.8	17.6	21.6	8	>32
	Ceftazidime	39.2	19.6	41.2	16	>32
	Ceftriaxone	11.8	19.6	68.6	64	>64
	Imipenem	100	0	0	0.25	1
	Levofloxacin	47.1	2	51	8	>8
	Minocycline	72	14	14	4	16
	Pip-Tazo	94.1	3.9	2	4	8
<i>E. aerogenes</i> (n=30)	Tigecycline	100	0	0	0.5	0.5
	Amikacin	100	0	0	2	4
	Amox-Clav	3.3	0	96.7	>32	>32
	Ampicillin	0	0	100	>32	>32
	Cefepime	90	0	10	≤0.5	4
	Ceftazidime	70	10	20	>8	>32
	Ceftriaxone	86.7	6.7	6.7	0.12	16
	Imipenem	96.7	0	3.3	0.5	1
	Levofloxacin	93.3	3.3	3.3	0.06	0.5
	Minocycline	96.7	0	3.3	2	4
	Pip-Tazo	86.7	13.3	0	2	32
<i>E. cloacae</i> (n=70)	Tigecycline	97.1	0	2.9	0.5	1
	Amikacin	97.1	0	2.9	2	4
	Amox-Clav	4.3	1.4	94.3	>32	>32
	Ampicillin	5.7	7.1	87.1	>32	>32
	Cefepime	92.9	0	7.1	≤0.5	8
	Ceftazidime	65.7	8.6	25.7	>8	>32
	Ceftriaxone	67.1	7.1	25.7	0.25	>64
	Imipenem	98.6	1.4	0	0.5	1
	Levofloxacin	91.4	4.3	4.3	0.03	2
	Minocycline	82.9	5.7	11.4	4	16
	Pip-Tazo	75.7	21.4	2.9	2	64
<i>S. marcescens</i> (n=50)	Tigecycline	100	0	0	1	1
	Amikacin	100	0	0	2	4
	Amox-Clav	9	8	84	>32	>32
	Ampicillin	12	8	80	>32	>32
	Cefepime	100	0	0	≤0.5	≤0.5
	Ceftazidime	96	0	4	>8	>8
	Ceftriaxone	98	2	0	0.25	4
	Imipenem	100	0	0	0.5	1
	Levofloxacin	100	0	0	0.12	0.25
	Minocycline	92	2	2	2	4
	Pip-Tazo	88	2	0	1	4

<sup>a</sup> Only species with n > 20 are represented.

<sup>b</sup> Breakpoints as defined by NCCLS where available (M100-S14), 2004. Tigecycline breakpoints defined as: susceptible ≤ 2; intermediate = 4; and resistant ≥ 8

Table 2. In Vitro Activity of Tigecycline and Comparative Agents against *A. baumannii* and *P. aeruginosa*

Organism Name <sup>a</sup>	Drug <sup>b</sup>	MIC (mcg/mL)				
		%SUS	%INT	%RES	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>A. baumannii</i> (n=75)	Tigecycline	97.3	2.7	0	0.25	1
	Amikacin	61.3	0	38.7	8	>64
	Amox-Clav	na	na	na	32	>32
	Ampicillin	na	na	na	>32	>32
	Cefepime	45.3	10.7	44	16	>32
	Ceftazidime	46.7	4	49.3	16	>32
	Ceftriaxone	29.3	21.3	49.3	32	>64
	Imipenem	68	2.7	29.3	0.5	>16
	Levofloxacin	57.3	34	18.7	2	8
	Minocycline	96	4	0	≤0.5	4
	Pip-Tazo	66.7	0	33.3	8	>128
<i>P. aeruginosa</i> (n=117)	Tigecycline	4.3	10.3	85.5	8	>16
	Amikacin	85.5	6	8.5	4	32
	Amox-Clav	na	na	na	>32	>32
	Ampicillin	na	na	na	>32	>32
	Cefepime	71.8	10.3	17.9	8	>32
	Ceftazidime	70.9	5.1	23.9	>8	>32
	Ceftriaxone	10.3	16.4	73.3	>64	>64
	Imipenem	86.3	3.4	10.3	1	16
	Levofloxacin	82.4	6	31.6	1	>8
	Minocycline	3.4	8.5	88	>16	>16
	Pip-Tazo	88.9	0	11.1	4	128

<sup>a</sup> Only species with n > 20 are represented.

<sup>b</sup> Breakpoints as defined by NCCLS where available (M100-S14), 2004. Tigecycline breakpoints defined as: susceptible ≤ 2; intermediate = 4; and resistant ≥ 8

Table 3. In Vitro Activity of Tigecycline and Comparative Agents against Gram Positive Pathogens

Organism Name	Drug <sup>a</sup>	MIC (mcg/mL)				
		%SUS	%INT	%RES	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>S. aureus</i> (n=150)	Tigecycline	100	0	0	0.12	0.25
	Amox-Clav	74.7	0	25.3	1	>8
	Ampicillin	8.7	0	91.3	8	>16
	Ceftriaxone	64	7.3	28.7	4	>64
	Imipenem	80	2	18	0.25	>16
	Levofloxacin	64	15.3	20.7	0.25	16
	Linezolid	100	0	0	2	4
	Minocycline	88	10	2	≤0.25	8
	Penicillin	8	0	92	>8	>8
	Pip-Tazo	74	0	26	1	>16