

Tigecycline Evaluation Surveillance Trial (T.E.S.T.) Program - In Vitro Antibacterial Activity Against Selected Species of *Enterobacteriaceae* from the Pacific Rim

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REVISED ABSTRACT

Background: Tigecycline, the first of a new class of glycylcyclines in clinical trials, has been shown to have potent activity against most species of *Enterobacteriaceae*. 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 members of *Enterobacteriaceae* collected from hospitals throughout Asia and the Pacific Rim. **Methods:** Minimum Inhibitory Concentration (MICs) of 402 isolates collected throughout 2004 were determined by broth microdilution and interpreted according to CLSI guidelines. **Results:** All the broad-spectrum antimicrobials tested in this study, with exception of ampicillin and amoxicillin/clavulanic acid, were highly active against *Enterobacteriaceae* and demonstrated susceptible rates above 75%. Tigecycline's activity was similar to imipenem presenting MIC₅₀/MIC₉₀ of 0.5/1 mcg/mL. The frequency of ESBL production among *E. coli* and *K. pneumoniae* was 16.4% and 27.8%, respectively. Tigecycline successfully inhibited 98% *E. coli* and *K. pneumoniae* ESBL producers at a MIC of 2 mcg/ml. Approximately 16% of *Enterobacter* spp. and *Serratia* spp. were resistant to third generation cephalosporins suggestive of AmpC-type resistance. Tigecycline was successful in inhibiting the >97% of these isolates at a MIC of 2 mcg/mL. **Conclusion:** Tigecycline's activity was comparable to the activities of broad spectrum antimicrobials and highly effective against ESBL and AmpC producing strains. Tigecycline's activity was comparable to imipenem which is often considered as last therapeutic option for the treatment of serious nosocomial infections caused by this class of organisms. The presented data suggest that tigecycline may be an effective therapeutic option against multidrug-resistant nosocomial *Enterobacteriaceae* including those with documented resistant phenotypes.

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 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 beta-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 Europe.

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.
- 402 clinical isolates were collected and tested between January 2004 – December 2004 from 6 study centers in Australia, China, India, Pakistan, Philippines and Singapore.
- Escherichia coli* and *Klebsiella pneumoniae* were screened and confirmed for ESBL activity according to CLSI guidelines (Table 2A, M100-S14) [13].

RESULTS

Table 1. List of countries and number of isolates contributed to T.E.S.T. program

Country	Total N
Australia	84
China	84
India	68
Philippines	84
Singapore	82
Total	402

Table 2. *In vitro* activity of tigecycline and comparative agents against 402 strains of *Enterobacteriaceae*

Organism (n)	Drug	%Sus	%Int	%Res	MIC (mcg/mL)	
					MIC ₅₀	MIC ₉₀
<i>Enterobacteriaceae</i> (n=402)	Tigecycline	98.8	0.7	0.5	0.5	1
	Amikacin	97.3	0	2.7	2	4
	Amox-Clav	41	14.4	44.5	16	>32
	Ampicillin	12.7	4.7	82.6	>32	>32
	Cefepime	91.3	3.2	5.5	0.5	8
	Ceftazidime	80.6	6	13.4	8	32
	Ceftriaxone	75.4	7	17.7	0.12	>64
	Imipenem	99.5	0.2	0.2	0.5	1
	Levofloxacin	80.3	2.5	17.2	0.06	>8
	Minocycline	81.3	10.2	8.5	2	8
Pip-Tazo	91	6.2	2.7	2	16	

^a 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 comparators against 402 strains of *Enterobacteriaceae* showing frequency distribution and cumulative percent inhibited (%) at each MIC (mcg/ml)

	MIC (mcg/ml)												
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
Tigecycline	14	67	100	153	48	15	1	2					
Amikacin	3.5	20.1	45	83.1	95	98.4	99.5	100					
Amox-Clav													
Ampicillin													
Cefepime													
Ceftazidime													
Ceftriaxone													
Imipenem													
Levofloxacin													
Minocycline													
Pip-Tazo													

Table 4. *In vitro* activity of tigecycline and comparative agents against selected representatives of *Enterobacteriaceae*

Organism (n) ^a	Drug ^b	%Sus	%Int	%Res	MIC (mcg/mL)	
					MIC ₅₀	MIC ₉₀
<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=69)	Tigecycline	97.1	0	2.9	0.5	1
	Amikacin	97.1	0	2.9	2	4
	Amox-Clav	4.3	1.4	94.2	>32	>32
	Ampicillin	5.8	7.2	87	>32	>32
	Cefepime	92.8	0	7.2	0.5	8
	Ceftazidime	66.7	8.7	24.6	8	>32
	Ceftriaxone	68.1	7.2	24.6	0.25	>64
	Imipenem	98.6	1.4	0	0.5	1
	Levofloxacin	91.3	4.3	4.3	0.03	2
	Tigecycline	82.6	5.8	11.6	4	>16
Pip-Tazo	76.8	20.3	2.9	2	64	
<i>E. coli</i> (n=122)	Tigecycline	100	0	0	0.12	0.25
	Amikacin	96.7	0	3.3	2	8
	Amox-Clav	66.4	27.9	5.7	8	16
	Ampicillin	28.7	0	71.3	>32	>32
	Cefepime	86.9	6.6	6.6	0.5	16
	Ceftazidime	88.5	5.7	5.7	8	16
	Ceftriaxone	72.1	6.6	21.3	≤0.06	>64
	Imipenem	100	0	0	0.25	0.5
	Levofloxacin	57.4	4.1	38.5	0.5	>8
	Minocycline	66.4	23	10.7	2	16
Pip-Tazo	98.4	0.8	0.8	1	4	
<i>K. oxytoca</i> (n=16)	Tigecycline	100	0	0	0.25	1
	Amikacin	100	0	0	2	4
	Amox-Clav	62.5	0	37.5	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	87.5	12.5	0	≤0.06	16
	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	75	0	25	2	>128	

- ESBL activity was confirmed by testing the following antibiotic disks: cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10µg), and ceftazidime (30µg), ceftazidime/clavulanic acid (30/10µg). 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 manufactures 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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<i>K. pneumoniae</i> (n=108)	Drug	%Sus	%Int	%Res	MIC (mcg/mL)	
					MIC ₅₀	MIC ₉₀
<i>K. pneumoniae</i> (n=108)	Tigecycline	97.2	2.8	0	0.5	1
	Amikacin	95.4	0	4.6	1	4
	Amox-Clav	61.1	17.6	21.3	4	>32
	Ampicillin	5.6	6.5	88	>32	>32
	Cefepime	89.8	4.6	5.6	0.5	16
	Ceftazidime	75.9	6.5	17.6	8	>32
	Ceftriaxone	69.4	9.3	21.3	0.12	>64
	Imipenem	100	0	0	0.5	1
	Levofloxacin	84.3	0.9	14.8	0.06	8
	Minocycline	84.3	5.6	10.2	2	16
Pip-Tazo	92.6	3.7	3.7	2	8	
<i>Serratia</i> spp (n=50)	Tigecycline	100	0	0	1	1
	Amikacin	100	0	0	2	4
	Amox-Clav	8	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	6	2	2	4
Pip-Tazo	98	2	0	1	4	

^a Only species with n ≥ 20 are represented.

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

Table 5. *In vitro* activity of tigecycline and comparative agents against extended-spectrum beta-lactamase producing *Enterobacteriaceae*

Organism (n)	Drug ^a	%Sus	%Int	%Res	MIC (mcg/mL)	
					MIC ₅₀	MIC ₉₀
<i>E. coli</i> (n=20)	Tigecycline	100	0	0	0.25	0.5
	Amikacin	95	0	5	2	4
	Amox-Clav	30	70	0	16	16
	Ampicillin	0	0	100	>32	>32
	Cefepime	50	30	20	8	>32
	Ceftazidime	45	30	25	16	32
	Ceftriaxone	5	20	75	>64	>64
	Imipenem	100	0	0	0.25	0.5
	Levofloxacin	60	0	80	>8	>8
	Minocycline	25	25	10	4	8
Pip-Tazo	100	0	0	2	8	
<i>K. pneumoniae</i> (n=30)	Tigecycline	96.7	3.3	0	0.5	1
	Amikacin	86.7	0	13.3	2	>64
	Amox-Clav	23.3	50	26.7	16	>32
	Ampicillin	3.3	0	96.7	>32	>32
	Cefepime	70	10	20	4	>32
	Ceftazidime	36.7	13.3	50	16	>32
	Ceftriaxone	16.7	20	63.3	64	>64
	Imipenem	100	0	0	0.5	1
	Levofloxacin	63.3	3.3	33.3	1	>8