

# 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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#### 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 MIC<sub>50</sub>/MIC<sub>90</sub> 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 MIC<sub>50</sub>/MIC<sub>90</sub> 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<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]. 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 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  $\geq 5$  mm in the inhibition zone of the combination disk when compared to that of the cephalosporin alone: cefotaxime/clavulanic acid - cefotaxime  $\geq 5$  mm or ceftazidime/clavulanic acid - ceftazidime  $\geq 5$  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  $\leq 2$ ; intermediate = 4; and resistant  $\geq 8$ .
- 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).

#### REFERENCES

- Sum, P.E. and P. Petersen. Synthesis and structure-activity relationship of novel glycylcycline derivatives leading to the discovery of GAR-936. Bioorg Med Chem Lett, 1999, 9(10): p. 1459-62.
- Abbanat, D., M. Macielag, and K. Bush. Novel antibacterial agents for the treatment of serious Gram-positive infections. Expert Opin Investig Drugs, 2003, 12(3): p. 379-99.
- Betriu, C., et al., In vitro activities of tigecycline (GAR-936) against recently isolated clinical bacteria in Spain. Antimicrob Agents Chemother, 2002, 46(3): p. 892-5.
- Gales, A.C. and R.N. Jones. Antimicrobial activity and spectrum of the new glycylcycline, GAR-936 tested against 1,203 recent clinical bacterial isolates. Diagn Microbiol Infect Dis, 2000, 36(1): p. 19-36.
- Henwood, C.J., et al., Antibiotic resistance among clinical isolates of *Acinetobacter* in the UK, and in vitro evaluation of tigecycline (GAR-936). J Antimicrob Chemother, 2002, 49(3): p. 479-87.
- Chopra, I., New developments in tetracycline antibiotics: glycylcyclines and tetracycline efflux pump inhibitors. Drug Resist Updat, 2002, 5(3-4): p. 119-25.
- Projan, S.J., Preclinical pharmacology of GAR-936, a novel glycylcycline antibacterial agent. Pharmacotherapy, 2000, 20(9 Pt 2): p. 219S-223S; discussion 224S-228S.
- Biedenbach, D.J., M.L. Beach, and R.N. Jones. In vitro antimicrobial activity of GAR-936 tested against antibiotic-resistant gram-positive blood stream infection isolates and strains producing extended-spectrum beta-lactamases. Diagn Microbiol Infect Dis, 2001, 40(4): p. 173-7.
- Patel, R., et al., In vitro activity of GAR-936 against vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae*. Diagn Microbiol Infect Dis, 2000, 38(3): p. 177-9.
- Petersen, P.J., et al., In vitro and in vivo antibacterial activities of a novel glycylcycline, the 9-t-butylglyclamido derivative of minocycline (GAR-936). Antimicrob Agents Chemother, 1999, 43(4): p. 738-44.
- Petersen, P.J., et al., In vitro and in vivo activities of tigecycline (GAR-936), daptomycin, and comparative antimicrobial agents against glycopeptide-intermediate *Staphylococcus aureus* and other resistant gram-positive pathogens. Antimicrob Agents Chemother, 2002, 46(8): p. 2595-601.
- National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Information Supplement. NCCLS document M100-S12. Wayne, PA, 2002.
- National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Information Supplement. NCCLS document M100-S14. Wayne, PA, 2004.

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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 <sub>50</sub>	MIC <sub>90</sub>	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	$\leq 0.5$	1	0.5->32
	Ceftazidime	$\leq 8$	16	<8->32
	Ceftriaxone	$\leq 0.06$	8	<0.06->64
Imipenem	0.5	1	0.12->16	
Levofloxacin	0.06	8	<0.008->8	
Minocycline	2	8	<0.5->128	
Pip-Tazo	1	16	<0.06->128	

Table 2. In vitro activity of tigecycline and comparators against 5,012 strains 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	$\leq 8$	8	>8	16	>16	32	>32	64	>64	128	>128
Tigecycline	9	279	1157	1243	1430	518	188	118	30															
Amikacin	0.02	5.7	236	54.4	83	93.3	97	93.4	100															
Amox-Clav	1				1.5	30	79.3	92.7	220	95	46	7	1											
Cefepime	0				0.6	49	32.5	48.9	61.6	68.9	75.7	100												
Ceftazidime					438.6	2	159	117	104	71	37	41	82											
Ceftriaxone					87.8	87.8	91	93.3	95.4	98.8	97.5	98.4	100											
Imipenem									446.5	14	55	88	350											
Levofloxacin									89.1	89.4	91.3	93	100											
Minocycline																								
Pip-Tazo																								

Table 3. In vitro activity of tigecycline and comparators against 87 strains of ESBL producing *E. coli*, *K. pneumoniae* and *K. oxytoca* showing cumulative percent inhibited (%) at each MIC (mcg/ml).

MIC	0.03	0.06	0.12	0.25	$\leq 0.5$	0.5	1	2	4	$\leq 8$	8	>8	16	>16	32	>32	64	>64	128	>128				
Tigecycline	9	13	24	23	13	4																		
Amikacin																								
Amox-Clav																								
Cefepime																								
Ceftazidime																								
Ceftriaxone																								
Imipenem																								
Levofloxacin																								
Minocycline																								
Pip-Tazo																								

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

Organism Name	Drug <sup>a</sup>	%SUS	%INT	%RES	MICs (mcg/mL)	
					MIC <sub>50</sub>	MIC <sub>90</sub>
<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	$\leq 0.5$	1
	Ceftazidime	84.5	6.2	9.3	$\leq 8$	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	$\leq 0.5$	4
	Ceftazidime	77.7	3.3	19	$\leq 8$	>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
<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	$\leq 0.5$	$\leq 0.5$
	Ceftazidime	95.2	1.2	3.6	$\leq 8$	$\leq 8$
	Ceftriaxone	96.3	1.3	2.4	$\leq 0.06$	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	2
	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	$\leq 0.5$	2
	Ceftazidime	87.1	1.4	11.5	$\leq 8$	>32
	Ceftriaxone	90.7	4	5.3	$\leq 0.06$	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	0.5
	Amikacin	100	0	0	2	4
	Amox-Clav	86.8	2.8	10.4	2	32
	Cefepime	100	0	0	$\leq 0.5$	1
	Ceftazidime	94.3	0	5.7	$\leq 8$	$\leq 8$
	Ceftriaxone	95.3	3.8	0.9	$\leq 0.06$	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	2
	Amikacin	100	0	0	2	4
	Amox-Clav	2.3	1.4	96.3	>32	>32
	Cefepime	96.6	1.7	1.7	$\leq 0.5$	1
	Ceftazidime	91.4	3.1	5.5	$\leq 8$	$\leq 8$
	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

<sup>a</sup>Breakpoints as defined by NCCLS (M100-S14), 2004. Tigecycline breakpoints defined as: susceptible  $\leq 2$ ; intermediate = 4; and resistant  $\geq 8$

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

Organism Name	Drug <sup>a</sup>	%SUS	%INT	%RES	MICs (mcg/mL)	
					MIC <sub>50</sub>	MIC <sub>90</sub>
<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
	Cefepime	53.3	0	46.7	8	>32
	Ceftazidime	26.7	13.3	60	32	>32
	Ceftriaxone	26.7	13.3	60	>64	>64
	Imipenem	100	0	0	0.25	1
	Levofloxacin	6.7	6.6	86.7	>8	>8
	Minocycline	73.3	0	26.7	2	>16
	Pip/Tazo	66.7	6.6	26.7	16	>128
<i>Klebsiella pneumoniae</i> , ESBL (n=68)	Tigecycline	92.6	5.9	1.5	1	2