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Tigecycline Evaluation Surveillance Trial (T.E.S.T.) - In Vitro Antibacterial Activity Against Selected Species of Enterobacteriaceae

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REVISED ABSTRACT

Background: Tigecycline (GAR-936), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent activity against most species of *Enterobacteriaceae*. The T.E.S.T. determined the in vitro activity of tigecycline compared to amoxicillin/clav, imipenem, cefepime, ceftazidime, ceftriaxone and levofloxacin against *Escherichia coli, Enterobacter* spp. and *Klebsiella* spp. from multi-national hospitals. **Methods:** A total of 1,031 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Isolates were collected between January 2004 – September 2004. MIC's were determined by the local laboratory using broth microdilution panels from Dade Microscan according to NCCLS guidelines and manufacturer's instructions. **Results:** Tigecycline demonstrated a MIC_{50} / MIC_{90} of 0.5/1 mcg/ml against all strains of *Enterobacteriaceae* that was equivalent to imipenem. The MIC_{90} of 1 for tigecycline was 8 fold lower than cefepime and levofloxacin and ≥64 fold lower than amoxicillin-clav, ceftazidime and ceftriaxone. Tigecycline inhibited >98% of all *E. coli* and *K. pneumoniae* ESBL producers at an MIC of 2 mcg/ml. **Conclusion:** Tigecycline is an effective antimicrobial agent against the selected Enterobacteriaceae in this study with low MICs against both ESBL and non-ESBL producing strains.

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]. The MIC_{90} values for pseudomonal isolates are generally elevated, in the range of 8-16 mcg/ml [10, 12]. The pharmacokinetics of parenteral tigecycline is linear with a mean half-life of 36 hours and a maximum serum concentration (C_{MAX}) of a 300mg dose infused over 1 hour of 2.8 mcg/ml [13,14].

This study compared the activity of tigecycline with other agents against Enterobacteriaceae including *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* from hospitals throughout Europe and North America.

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 – September 2004 from 20 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). 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.
- Isolates were identified to genus and species at each site by the local laboratory. Isolates were tested 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).

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RESULTS

Results are shown in the following tables.

Table 1. In Vitro Activity (mcg/ml) of Tigecycline and Comparative Agents Against 1661 Isolates of *Enterobacteriacae*.

Organism Name	Drug	MIC (mcg/mL) MIC_{50}	MIC_{90}	Range	
Enterobacteriaceae (n=1661)	Tigecycline	0.25	1	0.03	16
	Amikacin	2	4	<0.5	>64
	Amox/Clav	8	>32	0.5	>32
	Ampicillin	>32	>32	≤0.5	>32
	Cefepime	<0.5	2	<0.5	>32
	Ceftazidime	≤8	32	≤8	>32
	Ceftriaxone	≤0.06	16	≤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	2	32	≤0.06	>128

Table 2. In Vitro Activity (mcg/ml) of Tigecycline and Comparative Agents Against 1661 Isolates of *Enterobacter aerogenes, Enterobacter cloacae, E. coli, K. pneumoniae and K. oxytoca.*

Organism Name	Drug	%SUS*	%INT	%RES	MIC (mcg/mL) MIC_{50}	MIC_{90}
Enterobacter aerogenes (n=169)	Tigecycline	97.7	0.8	1.5	0.5	1
	Amikacin	100	0	0	2	4
	Amox/Clav	10	3.1	86.9	>32	>32
	Ampicillin	4.6	5.4	90	>32	>32
	Cefepime	96.9	1.5	1.5	≤0.5	2
	Ceftazidime	82.3	5.4	12.3	≤8	32
	Ceftriaxone	90.8	5.4	3.8	0.12	8
	Imipenem	99.2	0	0.8	1	2
	Levofloxacin	90	3.8	6.2	0.06	2
	Minocycline	89.2	6.9	3.8	2	8
	Pip/Tazo	85.4	7.7	6.9	2	32
Enterobacter cloacae (n=390)	Tigecycline	93	5.3	1.7	0.5	2
	Amikacin	99.7	0	0.3	2	2
	Amox/Clav	3	1	96	>32	>32
	Ampicillin	3.7	3	93.3	>32	>32
	Cefepime	93.7	2.3	4	≤0.5	4
	Ceftazidime	68.3	6.7	25	≤8	>32
	Ceftriaxone	73.7	11.7	14.7	0.5	64
	Imipenem	99.7	0	0.3	0.5	1
	Levofloxacin	90.3	2.7	7	0.03	2
	Minocycline	85	6.3	8.7	4	8
	Pip/Tazo	77.3	12.3	10.3	2	128
Escherichia coli (n=549)	Tigecycline	100	0	0	0.12	0.25
	Amikacin	99.3	0.5	0	2	4
	Amox/Clav	77.1	13.9	8.7	4	16
	Ampicillin	47.3	0.2	52.2	>32	>32
	Cefepime	97.9	0.2	1.7	≤0.5	≤0.5
	Ceftazidime	95.7	0.9	3.1	≤8	≤8
	Ceftriaxone	96.2	1.4	2.1	≤0.06	0.12
	Imipenem	99.8	0	0	0.25	0.5
	Levofloxacin	77.5	0.7	21.5	0.03	>8
	Minocycline	84.4	8.3	7.1	1	8
	Pip/Tazo	94.6	1.7	3.5	1	4
Klebsiella pneumoniae (n=451)	Tigecycline	94.8	4	1.2	0.5	2
	Amikacin	98.8	0.9	0.3	1	4
	Amox/Clav	86.7	6.9	6.3	2	16
	Ampicillin	3.7	16.4	79.8	>32	>32
	Cefepime	96.5	1.2	2.3	≤0.5	2
	Ceftazidime	89.6	0.9	9.5	≤8	16
	Ceftriaxone	91.9	4.3	3.7	≤0.06	4
	Imipenem	98.3	0.6	1.2	0.5	1
	Levofloxacin	91.1	1.7	7.2	0.06	2
	Minocycline	84.4	5.2	10.4	2	16
	Pip/Tazo	93.4	1.4	5.2	2	16
Klebsiella oxytoca (n=103)	Tigecycline	98.7	0	1.3	0.25	0.5
	Amikacin	98.7	1.3	0	2	4
	Amox/Clav	82.3	7.6	10.1	2	32
	Ampicillin	5.1	7.6	87.3	>32	>32
	Cefepime	94.9	0	5.1	≤0.5	4
	Ceftazidime	93.7	1.3	5.1	≤8	≤8
	Ceftriaxone	87.3	10.1	2.5	≤0.06	16
	Imipenem	100	0	0	0.5	0.5
	Levofloxacin	98.7	1.3	0	0.03	1
	Minocycline	93.7	1.3	5.1	1	4
	Pip/Tazo	87.3	1.3	11.4	1	128

* Breakpoints defined by the NCCLS 2004, document M100-S14; Tigecycline tentative breakpoints (in mcg/mL) defined as susceptible ≤ 2; intermediate = 4; resistant ≥ 8

Table 3. In Vitro MIC Values and Susceptibility Results of Tigecycline and Comparators Against 53 ESBL Producers and 947 Non-ESBL Producing *E. coli* and *K. pneumoniae*

Organism Name	Drug	%SUS*	%INT	%RES	MIC (mcg/mL) MIC_{50}	MIC_{90}
Escherichia coli, non-ESBL (n=540)	Tigecycline	100	0	0	0.12	0.25
	Amikacin	99.3	0.5	0	2	4
	Amox/Clav	77.9	13.5	8.4	4	16
	Ampicillin	48.1	0.2	51.4	>32	>32
	Cefepime	98.6	0.2	1	≤0.5	≤0.5
	Ceftazidime	96.9	0.7	2.2	≤8	≤8
	Ceftriaxone	97.4	1.2	1.2	<0.06	0.12
	Imipenem	99.8	0	0	0.25	0.5
	Levofloxacin	78.8	0.7	20.2	0.03	>8
	Minocycline	84.1	8.4	7.2	1	8
	Pip/Tazo	94.7	1.7	3.4	1	4
Escherichia coli, ESBL (n=9)	Tigecycline	100	0	0	0.25	0.5
	Amikacin	100	0	0	4	16
	Amox/Clav	28.6	42.9	28.6	16	32
	Ampicillin	0	0	100	>32	>32
	Cefepime	57.1	0	42.9	4	>32
	Ceftazidime	28.6	14.3	57.1	32	>32
	Ceftriaxone	28.6	14.3	57.1	64	>64
	Imipenem	100	0	0	0.25	1
	Levofloxacin	0	0	100	>8	>8
	Minocycline	100	0	0	1	4
	Pip/Tazo	85.7	0	14.3	8	>128
Klebsiella pneumoniae, non-ESBL (n=407)	Tigecycline	94.8	3.8	1.3	0.5	2
	Amikacin	99	0.6	0.3	1	2
	Amox/Clav	91.4	3.5	5.1	2	8
	Ampicillin	4.2	18.2	77.6	32	>32
	Cefepime	99.4	0	0.6	<0.5	<0.5
	Ceftazidime	98.1	0	1.9	≤8	≤8
	Ceftriaxone	98.7	0.6	0.6	<0.06	0.12
	Imipenem	98.7	0	1.3	0.5	1
	Levofloxacin	94.9	1.6	3.5	0.06	0.5
	Minocycline	85.9	5.1	8.9	2	8
	Pip/Tazo	95.5	1.3	3.2	2	8
Klebsiella pneumoniae, ESBL (n=44)	Tigecycline	94.1	5.9	0	0.5	2
	Amikacin	97.1	2.9	0	8	16
	Amox/Clav	44.1	38.2	17.6	16	32
	Ampicillin	0	0	100	>32	>32
	Cefepime	70.6	11.8	17.6	4	>32
	Ceftazidime	11.8	8.8	79.4	>32	>32
	Ceftriaxone	29.4	38.2	32.4	16	>64
	Imipenem	94.1	5.9	0	0.5	1
	Levofloxacin	55.9	2.9	41.2	2	>8
	Minocycline	70.6	5.9	23.5	4	16
	Pip/Tazo	73.5	2.9	23.5	8	>128
Klebsiella oxytoca, non-ESBL (n=92)	Tigecycline	98.6	0	1.4	0.25	0.5
	Amikacin	100	0	0	2	4
	Amox/Clav	87.3	5.6	7	2	16
	Ampicillin	5.6	8.5	85.9	>32	>32
	Cefepime	100	0	0	≤0.5	≤0.5
	Ceftazidime	100	0	0	<8	<8
	Ceftriaxone	97.2	1.4	1.4	≤0.06	0.5
	Imipenem	100	0	0	0.25	0.5
	Levofloxacin	98.6	1.4	0	0.03	0.12
	Minocycline	95.8	1.4	2.8	1	2

* Breakpoints defined by the NCCLS 2004, document M100-S14; Tigecycline tentative breakpoints (in mcg/mL) defined as susceptible ≤ 2; intermediate = 4; resistant ≥ 8

CONCLUSIONS

- Tigecycline inhibited 97% of all *Enterobacteriaceae* tested *in vitro* at an MIC of 2 µg/mL.
- Tigecycline's MIC_{90} of 1 µg/mL was equivalent to imipenem and cefepime and 8 to 64 fold better than the beta-lactams, beta-lactam/beta-lactamase inhibitor combinations and levofloxacin against all *Enterobacteriaceae* tested.
- Tigecycline demonstrated potent in vitro activity against both ESBL and non-ESBL producing *E. coli* and *K. pneumoniae* and *K. oxytoca*.
- The in vitro activity of tigecycline in this study suggests that tigecycline is a promising compound in the treatment of Gram-negative infections caused by selected *Enterobacteriaceae*.