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Tigecycline Evaluation Surveillance Trial (T.E.S.T.) Program - In Vitro Antibacterial Activity against Selected *Enterococcus* species from Asia and the Pacific Rim

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REVISED ABSTRACT

Background: Resistance to β-lactams and glycopeptides in enterococci was first recognized in the late 1980s, and since then has been a major challenge to clinicians and infection control. Tigecycline, the representative member of a new class of antimicrobials, glycylcyclines, has been shown to have potent activity against most commonly encountered species responsible for community and hospital infections. The T.E.S.T. program determined the in vitro activity of tigecycline compared to vancomycin, linezolid, ampicillin, imipenem, ceftriaxone, levofloxacin, minocycline, penicillin, and piperacillin/tazobactam against selected *Enterococcus* species collected from hospitals across Asia and the Pacific Rim. **Methods:** A total of 79 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Isolates were collected throughout 2004. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using broth microdilution panels and interpreted according to CLSI guidelines. **Results:** No vancomycin resistance was observed in the isolates tested. Overall, tigecycline presented the lowest MIC_{50}/MIC_{90} (0.12/0.25 mcg/mL) of all antimicrobial agents evaluated. Levofloxacin and minocycline had limited activities against *E. faecalis*. A high level of resistance to levofloxacin and minocycline was observed in *E. faecium* with non-susceptibility rates of 64.7% and 23.5%, respectively. Tigecycline presented the lowest MIC_{50}/MIC_{90} of 0.06/0.5 mcg/mL against this species. **Conclusion:** Tigecycline's low MIC_{50}/MIC_{90} values suggest that tigecycline may be an effective and reliable therapeutic option against nosocomial infections of enterococci.

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 and 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 [2-3].

Tigecycline has shown potent activity in animal models infected with selected strains of multi-drug resistant *Enterococcus faecium* and *Enterococcus faecalis* [4, 5] with diverse genotypes van-A, -B and -C [6]. Since current treatment options against vancomycin resistant *Enterococcus* spp. are largely limited to doxycycline, quinupristin/dalfopristin and linezolid, the activity of tigecycline was prospectively studied against a large geographically diverse population of enterococci in clinical settings.

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 6 study centers in Australia, China, India, Pakistan, Philippines and Singapore.
- Antimicrobial agents tested with concentrations (expressed in mcg/ml) were: piperacillin/tazobactam (0.25-16); levofloxacin (0.06-32); ceftriaxone (0.03-64); linezolid (0.5-8); minocycline (0.25-8); vancomycin (0.12-32); ampicillin (0.06-16); penicillin (0.06-8); tigecycline (0.008-16); imipenem (0.12-16). MIC interpretive criteria followed published guidelines established by the Clinical and Laboratory Standards Institute (CLSI) where applicable [7]. 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 followed CLSI guidelines using quality control organism *Enterococcus faecalis* ATCC 29212.
- 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).

ACKNOWLEDGEMENTS

This study was supported by a grant from Wyeth Pharmaceuticals. We gratefully acknowledge contributions to the T.E.S.T. program from the following participating institutions: P.D. Hinduja National Hospital, India; Research Institute for Tropical Medicine, Philippines and Peking Union Medical College Hospital, China; Singapore General Hospital, Singapore; Aga Khan University & Hospital, Pakistan; Flinders Medical Centre, Philippines.

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RESULTS

Table 1. *In vitro* activity of tigecycline and comparative agents against 79 strains of enterococci

					MIC (mcg/mL)	
Organism (n) [a]	**Drug [b]**	**%Sus**	**%Int**	**%Res**	**MIC_{50}**	**MIC_{90}**
Enterococcus spp (n=79)	Tigecycline	100	0	0	0.12	0.25
	Amox-Clav	na	na	na	1	8
	Ampicillin	91.1	0	8.9	1	4
	Ceftriaxone	na	na	na	>64	>64
	Imipenem	na	na	na	1	16
	Levofloxacin	54.4	2.5	43	2	32
	Linezolid	97.5	2.5	0	2	2
	Minocycline	40.5	38	21.5	8	>8
	Penicillin	83.5	0	16.5	2	>8
	Pip-Tazo	na	na	na	4	>16
	Vancomycin	100	0	0	1	2
Enterococcus faecalis (n=55)	Tigecycline	100	0	0	0.12	0.25
	Amox-Clav	na	na	na	0.5	2
	Ampicillin	100	0	0	1	2
	Ceftriaxone	na	na	na	>64	>64
	Imipenem	na	na	na	1	2
	Levofloxacin	60	0	40	1	32
	Linezolid	96.4	3.6	0	2	2
	Minocycline	36.4	45.5	18.2	8	>8
	Penicillin	94.5	0	5.5	2	8
	Pip-Tazo	na	na	na	4	8
	Vancomycin	100	0	0	1	2
Enterococcus faecium (n=17)	Tigecycline	100	0	0	0.06	0.5
	Amox-Clav	na	na	na	2	>8
	Ampicillin	58.8	0	41.2	4	>16
	Ceftriaxone	na	na	na	>64	>64
	Imipenem	na	na	na	16	>16
	Levofloxacin	23.5	11.8	64.7	16	>32
	Linezolid	100	0	0	1	2
	Minocycline	64.7	11.8	23.5	4	>8
	Penicillin	41.2	0	58.8	>8	>8
	Pip-Tazo	na	na	na	>16	>16
	Vancomycin	100	0	0	0.5	2

[a] Species with n's <10 are not shown.
[b] Breakpoints as defined by CLSI where applicable (M100-S14), 2004; na = not applicable.
Tigecycline breakpoints defined as: susceptible d" 2; intermediate = 4; and resistant e" 8.

Table 2. Frequency distribution (n) and cumulative percent inhibition (%) at each MIC (mcg/ml) for tigecycline and comparative agents against 56 *Enterococcus faecium*

	MIC (mcg/mL)												
n/Cum%	=0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Tigecycline	3	30	35	7	3	1							
	3.8	41.8	86.1	94.9	98.7	100							
Amox-Clav	2	1	2	3	30	24	8	1	1	7			
	2.5	3.8	6.3	10.1	48.1	78.5	88.6	89.9	91.1	100			
Ampicillin		2	2	5	10	36	11	6			7		
		2.5	5.1	11.4	24.1	69.6	83.5	91.1			100		
Ceftriaxone	2		1	3		3		1		3	3	3	60
	2.5		3.8	7.6		11.4		12.7		16.5	20.3	24.1	100
Imipenem			2	6	14	31	12	5		2	7		
			2.5	10.1	27.8	67.1	82.3	88.6		91.1	100		
Levofloxacin					10	29	4	2	7	7	13	7	
					12.7	49.4	54.4	57	65.8	74.7	91.1	100	
Linezolid					4	23	50	2					
					5.1	34.2	97.5	100					
Minocycline				14	1			17	30	17			
				17.7	19			40.5	78.5	100			
Penicillin		2	1	1	3	10	27	15	7	13			
		2.5	3.8	5.1	8.9	21.5	55.7	74.7	83.5	100			
Pip-Tazo				3		4	27	20	9	2	14		
				3.8		8.9	43	68.4	79.7	82.3	100		
Vancomycin				4	8	44	21	2					
				5.1	15.2	70.9	97.5	100					

CONCLUSIONS

- Tigecycline had the lowest MIC_{90} of all comparative agents against both *Enterococcus faecalis* (0.25 mcg/mL) and *Enterococcus faecium* (0.5 mcg/mL).
- No vancomycin-resistant isolates were encountered in the 79 consecutive clinical strains studied.
- Tigecycline's MIC_{90} of 0.25 mcg/mL was 8 to 16 fold lower than vancomycin, linezolid and ampicillin and 64 to 128 fold lower than minocycline and levofloxacin against all strains of enterococci.
- Tigecycline exhibits potent *in vitro* activity against *Enterococcus faecium* and *Enterococcus faecalis* but continued testing is warranted to document tigecycline activity against vancomycin-resistant strains in this geographic region.