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Tigecycline Evaluation Surveillance Trial (T.E.S.T.) - In Vitro Antibacterial Activity Against Selected Species of *Enterococcus* spp. - North America Data, 2004

T. Stevens[1], S. Bouchillon[1], J. Johnson[1], B. Johnson[1], D. Hoban[1], M. McCarthy[1], Char Gaylord[1], M. Dowzicky[2]

[1]International Health Management Associates, Schaumburg, IL, USA
[2]Wyeth Pharmaceuticals, Collegeville, PA, USA

IHMA, Inc.
2122 Palmer Dr.
Schaumburg, IL 60173
Tel: (847) 303-5003
Fax: (847) 303-5601
www.ihmainc.com

REVISED ABSTRACT

Background: Resistance to glycopeptides in enterococci was first recognized in the late 1980s, and since then has been a major challenge to clinicians and infection control. Tigecycline (GAR-936), a 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 *Enterococcus* spp. collected from hospitals in North America. **Methods**: A total of 730 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 with fresh media and interpreted according to NCCLS guidelines. **Results**: Of 534 *E. faecalis* evaluated, vancomycin resistance was observed in 26 (5.0%) isolates. These isolates were all susceptible to linezolid and tigecycline. Tigecycline presented the lowest MIC_{50}/MIC_{90} (0.06/0.12 mcg/ml) among all antimicrobial agents evaluated. As a typical profile of *E. faecalis*, fluoroquinolone (levofloxacin) and tetracycline (minocycline) had limited activities against this species. Among 196 *E. faecium*, 126 (64.3%) were resistant to vancomycin, of which five isolates were also resistant to linezolid. Tigecycline also presented the lowest MIC_{50}/MIC_{90} of 0.06/0.12 mcg/ml against this species. Interestingly, seven isolates of vancomycin susceptible *E. faecalis* and four *E. faecium* showed some degree of resistance to linezolid. No abnormal resistance phenotypes were observed in any other miscellaneous species of enterococci tested. **Conclusion**: The presented data suggest that tigecycline may be an effective and reliable therapeutic option against enterococci, including vancomycin- and linezolid-resistant 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 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 studied against a large population of enterococci from clinical settings in 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 and tested from January 2004 through December 2004 from 53 study centers in North America.
- 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 ≤ 2; intermediate = 4; and resistant ≥ 8.
- Isolates were identified to genus and species by the local laboratory. Each site tested their 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).

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RESULTS

Results are shown in the following tables.

Table 1. In vitro activity of tigecycline and comparative agents against 196 strains of *Enterococcus faecium*.

Organism Name	Drug [a]	%SUS	%INT	%RES	MICs (mcg/ml) MIC_{50}	MICs (mcg/ml) MIC_{90}
Enterococcus faecium	Tigecycline	100.0	0.0	0.0	0.06	0.12
(n=196)	Ampicillin	15.8	0.0	84.2	>16	>16
	Ceftriaxone	na	na	na	>64	>64
	Imipenem	na	na	na	>16	>16
	Levofloxacin	9.2	1.0	89.8	>32	>32
	Linezolid	93.9	2.0	4.1	2	2
	Minocycline	64.2	25.9	9.8	4	8
	Pip-Tazo	na	na	na	>16	>16
	Penicillin	13.3	0.0	86.7	>8	>8
	Vancomycin	34.2	1.5	64.3	>32	>32

[a] Breakpoints as defined by CLSI where applicable (M100-S14), 2004. na = not applicable. Tigecycline breakpoints defined as: susceptible ≤ 2; intermediate = 4; and resistant ≥ 8.

Table 2. In vitro activity of tigecycline and comparative agents against 126 strains of vancomycin-resistant *Enterococcus faecium*.

Organism Name	Drug [a]	%SUS	%INT	%RES	MICs (mcg/ml) MIC_{50}	MICs (mcg/ml) MIC_{90}
Enterococcus faecium	Tigecycline	100.0	0.0	0.0	0.06	0.12
(n=126)	Ampicillin	3.2	0.0	96.8	>16	>16
	Ceftriaxone	na	na	na	>64	>64
	Imipenem	na	na	na	>16	>16
	Levofloxacin	0.8	0.0	99.2	>32	>32
	Linezolid	93.7	2.4	4.0	2	2
	Minocycline	56.8	32.8	10.4	4	>8
	Pip-Tazo	na	na	na	>16	>16
	Penicillin	0.8	0.0	99.2	>8	>8
	Vancomycin	0.0	0.0	100.0	>32	>32

[a] Breakpoints as defined by CLSI where applicable (M100-S14), 2004. na = not applicable. Tigecycline breakpoints defined as: susceptible ≤ 2; intermediate = 4; and resistant ≥ 8.

Table 3. Frequency distribution (n) and cumulative percent inhibition (%) at each MIC (mcg/ml) for tigecycline and comparative agents against 196 *Enterococcus faecium*.

MIC	0.015	0.03	≤0.06	0.06	0.12	≤0.25	0.25	0.5	1	2	4	8	>8	16	>16	32	>32	64	>64
Tigecycline	2	80		82	30		2												
	1.0	41.8		83.7	99.0		100.0												
Ampicillin			1		1			1	17	6	2	3		3	162				
			0.5		1.0			1.5	10.2	13.3	14.3	15.8		17.3	100.0				
Ceftriaxone										3	2					5		8	178
										1.5	2.6					5.1		9.2	100.0
Imipenem							1	3	8	9	4	2			169				
							0.5	2.0	6.1	10.7	12.8	13.8			100.0				
Levofloxacin								4	6	8	2	3		4		20	149		
								2.0	5.1	9.2	10.2	11.7		13.8		24.0	100.0		
Linezolid									59	125	4	4	4						
									30.1	93.9	95.9	98.0	100.0						
Minocycline						81		7	3	3	30	50	19						
						42.0		45.6	47.2	48.7	64.2	90.2	100.0						
Penicillin					1				2	8	12	3	170						
					0.5				1.5	5.6	11.7	13.3	100.0						
Pip-Tazo									1	5	2	7		8	173				
									0.5	3.1	4.1	7.7		11.7	100.0				
Vancomycin							1	29	33	2	2	2		1		3	123		
							0.5	15.3	32.1	33.2	34.2	35.2		35.7		37.2	100.0		

Table 4. Frequency distribution (n) and cumulative percent inhibition (%) at each MIC (mcg/ml) for tigecycline and comparative agents against 126 strains of vancomycin resistant *Enterococcus faecium*.

MIC	0.015	0.03	0.06	0.12	<0.25	0.25	0.5	1	2	4	8	>8	16	>16	32	>32	64	>64
Tigecycline	1	51	57	16		1												
	0.8	41.3	86.5	99.2		100												
Ampicillin								2		1	1		1	121				
								1.6		2.4	3.2		4	100				
Ceftriaxone															1		1	124
															0.8		1.6	100
Imipenem								2		1	1			122				
								1.6		2.4	3.2			100				
Levofloxacin									1				3		16	106		
									0.8				3.2		15.9	100		
Linezolid								35	83	3	2	3						
								27.8	93.7	96	97.6	100						
Minocycline					48		3	1	2	17	41	13						
					38.4		40.8	41.6	43.2	56.8	89.6	100						
Penicillin										1		125						
										0.8		100						
Pip-Tazo											1		1	124				
											0.8		1.6	100				
Vancomycin															3	123		
															2.4	100		

Table 5. In vitro activity of tigecycline and comparative agents against 534 strains of *Enterococcus faecalis*.

Organism Name	Drug [a]	%SUS	%INT	%RES	MICs (mcg/ml) MIC_{50}	MICs (mcg/ml) MIC_{90}
Enterococcus faecalis	Tigecycline	100.0	0.0	0.0	0.06	0.12
(n=534)	Ampicillin	97.0	0.0	3.0	1	1
	Ceftriaxone	na	na	na	>64	>64
	Imipenem	na	na	na	1	1
	Levofloxacin	55.4	1.1	43.4	1	>32
	Linezolid	98.7	0.9	0.4	2	2
	Minocycline	40.1	47.1	12.8	8	>8
	Pip-Tazo	na	na	na	2	4
	Penicillin	96.1	0.0	3.9	2	4
	Vancomycin	94.6	0.6	4.9	1	2

[a] Breakpoints as defined by CLSI where applicable (M100-S14), 2004. na = not applicable. Tigecycline breakpoints defined as: susceptible ≤ 2; intermediate = 4; and resistant ≥ 8.

Table 6. In vitro activity of tigecycline and comparative agents against 26 strains of vancomycin-resistant *Enterococcus faecalis*.

Organism Name	Drug [a]	%SUS	%INT	%RES	MICs (mcg/ml) MIC_{50}	MICs (mcg/ml) MIC_{90}
Enterococcus faecalis	Tigecycline	100.0	0.0	0.0	0.06	0.12
(n=26)	Ampicillin	61.5	0.0	38.5	2	>16
	Ceftriaxone	na	na	na	>64	>64
	Imipenem	na	na	na	2	>16
	Levofloxacin	11.5	0.0	88.5	>32	>32
	Linezolid	100.0	0.0	0.0	2	2
	Minocycline	38.5	53.8	7.7	8	8
	Pip-Tazo	na	na	na	4	>16
	Penicillin	53.8	0.0	46.2	8	>8
	Vancomycin	0.0	0.0	100.0	>32	>32

[a] Breakpoints as defined by CLSI where applicable (M100-S14), 2004. na = not applicable. Tigecycline breakpoints defined as: susceptible ≤ 2; intermediate = 4; and resistant ≥ 8.

Table 7. Frequency distribution (n) and cumulative percent inhibition (%) at each MIC (mcg/ml) for tigecycline and comparative agents against 534 *Enterococcus faecalis*.

MIC	0.015	0.03	≤0.06	0.06	≤0.12	0.12	≤0.25	0.25	≤0.5	0.5	1	2	4	8	>8	16	>16	32	>32	64	>64
Tigecycline	3	24		243		236		26		1											
	0.6	5.1		50.7		94.9		99.8		100											
Ampicillin						2		4		143	332	30	3	4		3	13				
						0.4		1.1		27.9	90.1	95.7	96.3	97		97.6	100				
Ceftriaxone										1	4	4	1	2		20		39		72	391
										0.2	0.9	1.7	1.9	2.2		6		13.3		26.8	100
Imipenem					1			7		173	304	24	4	1		2	18				
					0.2			1.5		33.9	90.8	95.3	96.1	96.3		96.6	100				
Levofloxacin			1					2		95	181	17	6	4		36		73	119		
			0.2					0.6		18.4	52.2	55.4	56.6	57.3		64		77.7	100		
Linezolid									7		187	333	5	1	1						
									1.3		36.3	98.7	99.6	99.8	100						
Minocycline							121			13	7	13	56	247	67						
							23.1			25.6	26.9	29.4	40.1	87.2	100						
Penicillin						1		1		3	42	372	86	8	21						
						0.2		0.4		0.9	8.8	78.5	94.6	96.1	100						
Pip-Tazo										3	53	346	95	11		3	23				
										0.6	10.5	75.3	93.1	95.1		95.7	100				
Vancomycin					1					32	332	135	4	3				3	23		
					0.2					6.2	68.5	93.8	94.6	95.1				95.7	100		

Table 8. Frequency distribution (n) and cumulative percent inhibition (%) at each MIC (mcg/ml) for tigecycline and comparative agents against 26 vancomycin-resistant *Enterococcus faecalis*.

MIC	0.03	0.06	≤0.12	≤0.25	0.25	≤0.5	0.5	1	2	4	8	>8	16	>16	32	>32	64	>64
Tigecycline	3	13	8		2													
	11.5	61.5	92.3		100													
Ampicillin							3	9	2	1	1			10				
							11.5	46.2	53.8	57.7	61.5			100				
Ceftriaxone																	1	25
																	3.8	100
Imipenem							5	7	1	1			1	11				
							19.2	46.2	50	53.8			57.7	100				
Levofloxacin								2	1						9	14		
								7.7	11.5						46.2	100		
Linezolid						1		8	17									
						3.8		34.6	100									
Minocycline				4			2		2	2	14	2						
				15.4			23.1		30.8	38.5	92.3	100						
Penicillin								1	6	5	2	12						
								3.8	26.9	46.2	53.8	100						
Pip-Tazo								1	7	5			2	11				
								3.8	30.8	50			57.7	100				
Vancomycin															3	23		
															11.5	100		

CONCLUSIONS

- Tigecycline MIC_{90} of 0.12 mcg/ml against all study strains of *Enterococcus faecium* and *Enterococcus faecalis* was the lowest of all study drugs.
- Tigecycline MIC_{90}s against vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* were more than 16-fold lower than those of linezolid, minocycline or levofloxacin .
- Tigecycline exhibits potent in vitro activity against *Enterococcus faecium* and *Enterococcus faecalis* regardless of vancomycin phenotype.