

REVISED ABSTRACT

Objective: Glycylcyclines are a new class of antimicrobials that show promise of significant activity against many gram-positive pathogens including *S. aureus*. Tigecycline, a member of this new class of antimicrobials, has shown excellent activity against *Staphylococcus* spp. This study was initiated to evaluate the *in vitro* activity of tigecycline as compared with those of 10 comparator agents (ampicillin, penicillin, imipenem, ceftriaxone, levofloxacin, minocycline, vancomycin, linezolid, amoxicillin/clavulanic acid, and piperacillin-tazobactam) against *S. aureus* including methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) from study sites across Asia and the Pacific Rim as part of the T.E.S.T. program. **Methods:** A total of 140 clinical isolates were collected throughout 2004 and MICs were determined by each participating center using broth microdilution. All testing was performed and interpreted according to CLSI guidelines and manufacturer's instructions. **Results:** Among the 140 isolates, 23 (45.1%) were found to be resistant to methicillin (MRSA). Besides the expected cross resistance of MRSA isolates to imipenem, ceftriaxone, penicillin, ampicillin, amoxicillin/clavulanic acid, and piperacillin-tazobactam, a high rate of non-susceptibility (I+R) to levofloxacin (60.9%) was observed. No resistance was observed against vancomycin and linezolid. The MICs of tigecycline ranged from 0.06 to 0.5 mcg/mL against all strains of *S. aureus*. Tigecycline presented the lowest MIC₅₀/MIC₉₀ of 0.25/0.25 mcg/mL against MRSA strains, being several folds lower than all the comparator agents. The MSSA isolates showed the expected profile of high resistance to ampicillin and penicillin. Tigecycline's MIC₅₀/MIC₉₀ of 0.12/0.25 mcg/mL was also the lowest among all MSSA isolates. **Conclusion:** The inhibitory activity of tigecycline was satisfactory in all *S. aureus* tested regardless of methicillin phenotype. Tigecycline may be an effective and reliable therapeutic option against *S. aureus* regardless of degree or type of resistance.

INTRODUCTION

Tigecycline is the first 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].

While developed to provide activity against tetracycline- and multi-drug-resistant gram-positive pathogens, it has been demonstrated to possess significant broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative microorganisms [1, 3-5]. Tigecycline MIC₉₀ value of ≤ 0.5 mcg/mL have been demonstrated against methicillin-resistant *Staphylococcus aureus* (MRSA) [2, 4-6].

Tigecycline resistance is very infrequent and difficult to induce in the laboratory [7, 8] with a selection frequency observed at less than 10⁻⁹ [2, 3, 7]. Most tetracycline-resistant bacteria with either tetracycline efflux pumps or ribosomal protective features are sensitive to tigecycline [1-4, 6, 9-11]. The pharmacokinetics of parenteral tigecycline is linear with an unusually long half-life of 36 hours and a maximum serum concentration (C_{MAX}) of 2.8 mcg/mL from a 300 mg dose infused over 1 hour [12].

This study compared the activity of tigecycline with other agents against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) in laboratories from Australia, China, India, Pakistan, Philippines and Singapore..

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: amoxicillin/clavulanic acid (0.03-8); 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 [13]. 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. All MRSA and MSSA were confirmed by the central laboratory using oxacillin disk test (Oxoid).
- Quality control followed CLSI guidelines using quality control organism *Staphylococcus aureus* ATCC 29213.
- The collection and transporting 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

Table 1: *In vitro* activity of tigecycline and comparator agents against 140 Isolates of *Staphylococcus aureus*.

Organism (n)	Drug ^a	%Sus	%Int	%Res	MIC (mcg/mL)	
					MIC ₅₀	MIC ₉₀
<i>S. aureus</i> (n=140)	Tigecycline	100	0	0	0.12	0.25
	Amox-Clav	75.7	0	24.3	1	>8
	Ampicillin	7.9	0	92.1	8	>16
	Ceftriaxone	65	7.1	27.9	4	>64
	Imipenem	80	2.1	17.9	0.25	>16
	Levofloxacin	65	14.3	20.7	0.25	16
	Linezolid	100	0	0	2	4
	Minocycline	89.3	8.6	2.1	≤ 0.25	8
	Penicillin	7.1	0	92.9	>8	>8
	Pip-Tazo	75	0	25	1	>16
Vancomycin	100	0	0	1	1	
Methicillin-Susceptible <i>S. aureus</i> (n=85)	Tigecycline	100	0	0	0.12	0.25
Amox-Clav	96.5	0	3.5	0.5	2	
Ampicillin	12.9	0	87.1	4	>16	
Ceftriaxone	92.9	3.5	3.5	4	4	
Imipenem	97.6	1.2	1.2	0.25	0.25	
Levofloxacin	89.4	5.9	4.7	0.12	4	
Linezolid	100	0	0	2	4	
Minocycline	98.8	0	1.2	≤ 0.25	0.5	
Penicillin	11.8	0	88.2	4	>8	
Pip-Tazo	96.5	0	3.5	1	2	
Vancomycin	100	0	0	1	1	
Methicillin-Resistant <i>S. aureus</i> (n=55)	Tigecycline	100	0	0	0.12	0.5
Amox-Clav	43.6	0	56.4	>8	>8	
Ampicillin	0	0	100	>16	>16	
Ceftriaxone	21.8	12.7	65.5	>64	>64	
Imipenem	52.7	3.6	43.6	1	>16	
Levofloxacin	27.3	27.3	45.5	4	>32	
Linezolid	100	0	0	2	2	
Minocycline	74.5	21.8	3.6	2	8	
Penicillin	0	0	100	>8	>8	
Pip-Tazo	41.8	0	58.2	>16	>16	
Vancomycin	100	0	0	1	1	

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

Table 2. Frequency distribution (n) and cumulative percent inhibition (%) at each MIC (mcg/mL) for tigecycline and comparative agents against 85 methicillin-sensitive *Staphylococcus aureus*.

n/Cum%	MIC (mcg/mL) ^a											
	≤ 0.06	0.06	0.12	0.25	0.5	1	2	4	8	16	64	>64
Tigecycline	10	53	19	3								
Amox-Clav	11.8	74.1	96.5	100								
Ampicillin	5	4	9	32	31	4	2	1	2			
Ceftriaxone	5.9	10.6	12.9	25.9	32.9	45.9	57.6	72.9	82.4	100		
Imipenem					5	35	39		3			3
Levofloxacin	7	43	18	6	2	2		5	2	2		
Linezolid	8.2	58.8	80	87.1	89.4	95.3	97.6	100				
Minocycline					74	7		3		1		
Penicillin	10	11.8			87.1	95.3		98.8		100		
Pip-Tazo					2	9	10	5	12	8	29	
Vancomycin					14.1	24.7	36.5	42.4	56.5	65.9	100	
					2	37	35	5	2	1		3
					2.4	45.9	87.1	92.9	95.3	96.5	100	
					2	33	49	1				
					2.4	41.2	98.8	100				

^a Some \leq and $>$ values have been normalized by rounding up to the next highest MIC.

Table 3. Frequency distribution (n) and cumulative percent inhibition (%) at each MIC (mcg/mL) for tigecycline and comparative agents against 55 methicillin-resistant *Staphylococcus aureus*.

n/Cum%	MIC (mcg/mL) ^a											
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Tigecycline	6	23	17	9								
Amox-Clav	10.9	52.7	83.6	100								
Ampicillin					1.8	7.3	10.9	20	43.6	49.1	100	
Ceftriaxone					2	5.5	9.1	14.5	16.4	36.4	100	
Imipenem					3.6	3	1	7	6	1	4	32
Levofloxacin					5.9	47.1	92.9	96.5				100
Linezolid					5	19	2	2	1	2	4	20
Minocycline					9.1	43.6	47.3	50.9	52.7	56.4	63.6	100
Penicillin					8	7			15	10	7	2
Pip-Tazo					14.5	27.3			54.5	72.7	85.5	89.1
Vancomycin					17	34	4		30.9	92.7	100	
					24	43.6			6	11	12	2
									54.5	74.5	96.4	100
									2	4	1	47
									5.5	12.7	14.5	100
									1	3	2	30
									1.8	7.3	10.9	20
									17	34	4	
									30.9	92.7	100	

^a Some \leq and $>$ values have been normalized by rounding up to the next highest MIC.

CONCLUSIONS

- Tigecycline inhibited the growth of all MSSA and MRSA at a MIC ≤ 0.5 mcg/mL.
- Tigecycline demonstrates greater *in vitro* activity against MSSA and MRSA than levofloxacin, imipenem and the β -lactam antimicrobials.
- Tigecycline demonstrates *in vitro* activity comparable to commonly prescribed antimicrobial agents, linezolid and vancomycin, currently used for the treatment of serious staphylococcal nosocomial infections.
- Tigecycline appears to be promising agent in the treatment of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*.