

European In Vitro Antibacterial Activity of Tigecycline against Methicillin Resistant and Methicillin Sensitive *Staphylococcus aureus* Isolates from the Tigecycline Evaluation Surveillance Trial (T.E.S.T.) Program

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REVISED ABSTRACT

Background: Resistant gram-positive bacteria, especially *Staphylococcus aureus*, continues to be a therapeutic challenge for the clinician. Despite the introduction of new antimicrobials to treat this organism, Glycylcyclines are showing the promise of significant activity against many gram-positive pathogens including methicillin-resistant *S. aureus*. Tigecycline, the first glycylcycline to enter clinical trials, 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, amoxicillin-clavulanic acid, imipenem, ceftriaxone, levofloxacin, minocycline, vancomycin, linezolid, piperacillin-tazobactam) against *S. aureus* including methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) from 15 European centers in the T.E.S.T. program. **Methods:** A total of 366 clinical isolates were identified to the species level at each of participating sites and confirmed by the central laboratory. Isolates were collected throughout 2004. MICs were determined by each participating laboratory using custom broth microdilution panels from Dade Behring MicroScan. All testing was performed and interpreted according to CLSI guidelines and manufacturer's instructions. **Results:** Among the 366 isolates, 98 (26.8%) were found to be resistant to methicillin (MRSA). Besides the cross resistance of MRSA isolates to imipenem, ceftriaxone, penicillin, ampicillin and piperacillin/tazobactam, most of MRSA isolates were also non-susceptible to levofloxacin. No resistance was observed against vancomycin and linezolid. The MICs of tigecycline ranged from 0.06 to 0.5 mcg/mL for all isolates of *S. aureus*, and tigecycline presented the lowest MIC₅₀/MIC₉₀ of 0.12/0.25 mcg/ml against MRSA isolates, being several folds lower than all the comparator agents. The MSSA isolates showed the expected profile of high resistance to ampicillin and penicillin. Opposite to MRSA isolates, MSSA presented very little resistance to levofloxacin (5.6%). Tigecycline's MIC₅₀/MIC₉₀ of 0.12/0.12 was also the lowest among all MSSA isolates. **Conclusion:** The in vitro activity of tigecycline was comparable in all *S. aureus* tested regardless of methicillin susceptibility. Tigecycline's activity against MRSA was equivalent to commonly prescribed agents, linezolid and vancomycin, for the treatment of serious nosocomial infections.

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 broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative microorganisms [1,3-5]. Tigecycline MIC₉₀ value of <0.5 mcg/ml has 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 a 300 mg dose infused over 1 hour of 2.8 mcg/ml [12].

This study compared the activity of tigecycline with other agents against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) from hospitals across Europe.

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 15 study centers in 10 countries.
- 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: List of countries and number of investigative sites that contributed to T.E.S.T. program.

Country	Investigative Sites
France	2
Germany	4
Hungary	1
Italy	2
Latvia	1
Poland	1
Spain	1
Switzerland	1
The Netherlands	1
United Kingdom	1
Total	15

Table 2: In vitro activity of tigecycline and comparator agents against 366 Isolates of *Staphylococcus aureus*.

Oranism Name	Drug ^a	%S	%I	%R	MICs (mcg/mL)	
					MIC ₅₀	MIC ₉₀
<i>Staphylococcus aureus</i> (n=366)	Tigecycline	100	0	0	0.12	0.25
	Amox-Clav	82	0	18	1	>8
	Ampicillin	17.5	0	82.5	8	>16
	Ceftriaxone	76.2	7.4	16.4	4	>64
	Imipenem	86.3	0.5	13.2	0.25	>16
	Levofloxacin	73	7.6	19.4	0.12	8
	Linezolid	100	0	0	2	4
	Minocycline	99.2	0.8	0	≤0.25	0.5
	Pip-Tazo	83.3	0	16.7	1	>16
	Penicillin	14.8	0	85.2	8	>8
Vancomycin	100	0	0	1	1	
<i>Staphylococcus aureus</i> , MSSA (n=268)	Tigecycline	100	0	0	0.12	0.12
	Amox-Clav	99.6	0	0.4	0.5	1
	Ampicillin	23.5	0	76.5	4	>16
	Ceftriaxone	99.3	0.3	0.4	2	4
	Imipenem	99.6	0	0.4	0.25	0.25
	Levofloxacin	94.4	1.5	4.1	0.12	0.5
	Linezolid	100	0	0	2	4
	Minocycline	99.6	0.4	0	≤0.25	0.5
	Pip-Tazo	99.6	0	0.4	0.5	1
	Penicillin	19.9	0	80.1	4	>8
Vancomycin	100	0	0	1	1	

<i>Staphylococcus aureus</i> , MRSA (n=98)	Tigecycline	100	0	0	0.12	0.25
Amox-Clav	33.7	0	66.3	>8	>8	
Ampicillin	1	0	99	>16	>16	
Ceftriaxone	13.3	26.5	60.2	>64	>64	
Imipenem	50	2	48	8	>16	
Levofloxacin	14.3	24.5	61.2	8	16	
Linezolid	100	0	0	2	2	
Minocycline	98	2	0	≤0.25	4	
Pip-Tazo	38.8	0	61.2	>16	>16	
Penicillin	1	0	99	>8	>8	
Vancomycin	100	0	0	1	2	

^aBreakpoints as defined by NCCLS (M100-S14), 2004. 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 268 methicillin-sensitive *Staphylococcus aureus*.

MIC	≤0.008	≤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	
Tigecycline	1	52	190	24	1															
Amox-Clav	0.4	19.8	90.7	99.6	100															
Ampicillin	28	0.7	16.8	26.9	58.2	94.8	99.6													
Ceftriaxone	10.4		24	11	20	17	22	36	31											
Imipenem			1	1	2	15	129	115	4											
Levofloxacin	48	130	48.5	132	3	2														
Linezolid	17.9		133	59	9	4														
Minocycline			67.5	89.6	92.9	94.4	95.9	98.1	98.9											
Penicillin	45		8	85.1	14	15	17	17	29	30	92									
Pip-Tazo	16.9		19.9	25.1	30.7	37.1	43.4	54.3	65.5	100										
Vancomycin				39	14.6	59.3	94	98.1	99.6											

Table 4. Frequency distribution (n) and cumulative percent inhibition (%) at each MIC (mcg/mL) for tigecycline and comparative agents against 98 methicillin-resistant *Staphylococcus aureus*.

MIC	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	
Tigecycline	1	12	51	31	2	1														
Amox-Clav	1	13.3	65.3	96.9	99	100														
Ampicillin	1		2	1	7	10	13	12	53											
Ceftriaxone	1		1	5.1	7.1	10.2	17.3	31.6	100											
Imipenem	1		1	2	1	4	5	19	7											
Levofloxacin	2	8	2	31	5	1	3	2	7	40										
Linezolid	2		6	39.8	44.9	45.9	46.9	50	52	59.2	100									
Minocycline			2	2	2	24	32	21	6	1										
Penicillin	1		8.2	10.2	12.2	14.3	38.8	71.4	92.9	99	100									
Pip-Tazo	1		1	8	13	70	7													
Vancomycin				57	58.2	79.6	80.6	87.8	98	100										

CONCLUSIONS

- Tigecycline inhibited the growth of all MSSA and MRSA at a MIC ≤ 1 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*.