Multicenter Evaluation of Tigecycline: An In Vitro Update Against Clinical Pathogens from Japan (2006-2007)

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ABSTRACT

Background: Tigecycline, a novel glycylcycline class agent (first in class), has been utilized for serious multidrug-resistant (MDR) pathogen infections in several global locations for 1-3 years. This prospective in vitro multicenter trial evaluates nearly 4,000 pathogens from Japan (2006-2007).

Methods: A total of 3,902 isolates (19 medical centers) were processed by reference broth microdilution methods in a single central laboratory. CLSI methods were applied and breakpoints found in CLSI M100-S18 (2008) or USA-FDA Tygacil®; package insert were used. The most prevalent organisms were: *S. aureus* (SA; 1,198), *S. pneumoniae* (SPN; 459), *E. coli* and *Klebsiella* (199 each), CoNS (198), *Enterobacter* spp. (197), enterococci (195) and *Acinetobacter* spp. (193). All concurrent QC was acceptable.

Results: Against Gram-positive cocci, tigecycline $MIC_{50/90}$ values were: SA (0.12/0.5 mg/L), enterococci and CoNS (0.12/0.25), SPN (≤0.03/0.06), and ß-haemolytic (BHS) or viridans group streptococci (≤0.03/0.06); all susceptible except 3 MRSA (MIC at 1 mg/L). Among Enterobacteriaceae, tigecycline inhibited 100.0, 99.0 and 100.0% of *E. coli*, *Klebsiella* and *Enterobacter* spp. at ≤2 mg/L, respectively. *P. mirabilis* and *S. marcescens* were less susceptible to tigecycline (MIC_{90} range, 1 to 4 mg/L). *Acinetobacter* spp. (193) had MIC values ranging to 4 mg/L; 99.0% at ≤2 mg/L and 1.6% carbapenem-resistant. *P. aeruginosa* were generally tigecycline-resistant (MIC_{90}, >4 mg/L). 14.5% of group B were levofloxacin-resistant (clonal with multiple QRDR mutations in 7 sites). ESBL rates were only 2.0-6.5% in *E. coli* and *Klebsiella*, and fluoroquinolones and trimethoprim/sulfamethoxazole resistances were highest (20.1-21.1%) in *E. coli*.

	MIC of tigecycline		Cum. % inhibited at MIC (mg/L)[b]				
Organism (no. tested[a])	50%	90%	0.25	0.5	1	2	4
S. aureus, methicillin-R (584)	0.25	0.5	68.7	99.5	100.0	-	-
CoNS (198)	0.12	0.25	94.4	100.0	-	-	-
S. pneumoniae, penicillin-R (148)	≤0.03	0.06	100.0	-	-	-	-
E. coli (199)	0.12	0.25	98.0	100.0	100.0	100.0	-
Enterobacter spp. (197)	0.25	0.5	69.5	91.9	96.5	100.0	-
Klebsiella spp. (199)	0.25	0.5	73.4	94.5	96.0	99.9	100.0
Acinetobacter spp. (193)	0.25	1	82.4	89.6	98.5	99.0	100.0

a. *P. aeruginosa* (598 strains) had resistant level MIC_{50} results (4 mg/L), data not shown.
b. Underlined value is at USA-FDA breakpoint.

Conclusions: Tigecycline was observed to be active against nearly all tested species from Japanese medical centers for year 2003-2004 strains, and in this prospective sample report remains similarly active (2006-2007 isolates). Resistant subsets (MRSA, ESBL enteric bacilli, MDR *Acinetobacter* spp.) were generally inhibited at USA-FDA-susceptible breakpoints for tigecycline. Possible use of tigecycline for some serious MDR infections in Japan should be considered.

INTRODUCTION

Tigecycline, introduced in 2005, is the first glycylcycline to be used in human clinical practice worldwide. This semisynthetic agent was a derivative of minocycline that has a unique, bacteriostatic effect by binding to bacterial ribosomes in a manner preventing the two principal determinants of tetracycline resistance (efflux, ribosomal protection). Cross- or co-resistance of tigecycline with other drug classes among Gram-positive cocci, Enterobacteriaceae and *Acinetobacter* spp. is rare; therefore, potential applications in multidrug-resistant (MDR) species in Japan may facilitate improved clinical outcomes.

To evaluate a large collection of contemporary (2007) Gram-positive and -negative isolates, 19 medical centers in Japan were recruited to supplement an earlier (2003-2004) sample. The selection of the organisms was to focus on those species of greatest concern regarding emerging antimicrobial resistance patterns detected in Japan and worldwide (Infectious Disease Society of America). A central laboratory design (JMI Laboratories, North Liberty, IA, USA) was employed to assure data accuracy via use of rigid quality control (QC), concurrent reviews by experienced directors and follow-up molecular methods (epidemiology, mechanisms of resistance, etc.). Testing of newer agents and novel classes (tigecycline) was emphasized by applications of reference susceptibility testing methods of the Clinical and Laboratory Standards Institute (CLSI).

MATERIALS AND METHODS

Bacterial isolates. A total of 3,902 bacterial isolates collected during 2007 in 19 medical centers located in Japan were evaluated as part of the SENTRY Antimicrobial Surveillance Program. The isolates were consecutively collected from bloodstream infections, skin and soft tissue infections, urinary tract infections and pneumonia in hospitalized patients according to a common protocol. Only isolates from documented infections were included in the study. Species identification was confirmed by the central monitor using standard biochemical tests and the Vitek System (bioMerieux, Hazelwood, MO), when necessary.

Antimicrobial susceptibility testing. All isolates were susceptibility tested using the broth microdilution method as described by the CLSI (formerly NCCLS). Fresh cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, OH). Categorical interpretations for comparator antimicrobials were those found in M100-S18; breakpoints for tigecycline were those of the United States (US) Food and Drug Administration (FDA) or by Jones et al. (2007). QC was performed using *Escherichia coli* ATCC 25922, *S. aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853; all QC results were within specified ranges as published in M100-S18. Enterobacteriaceae with elevated MIC values (≥2 mg/L) for ceftazidime and/or ceftriaxone and/or aztreonam were considered as extended-spectrum ß-lactamase (ESBL)-producing phenotypes. *Acinetobacter* spp., with imipenem or meropenem MICs ≥8 mg/L, and Enterobacteriaceae with imipenem or meropenem MICs at ≥2 mg/L, were screened for metallo-ß-lactamase (MBL) enzymes and OXA-23, -24, and -58 enzymes by PCR. Enterobacteriaceae with an ertapenem MIC at >1 mg/L were screened for KPC-type serine carbapenemases. Organisms exhibiting unusual resistant antibiograms were also subjected to molecular analyses.

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RESULTS

- Nineteen medical centers in Japan participated in the Prospective Phase of this tigecycline study (2007). Each participant contributed 162-560 organisms or an average of 205 strains per site. The target number of sites was 20 at 200 organisms per site. 3,902 strains were forwarded for 97.55% compliance to protocol.
- Table 1 lists the occurrences of tigecycline MIC results for 15 organism groups tested by the CLSI reference method. The rates of susceptibility (where interpretive breakpoints exist) ranged from 79.7% (*P. mirabilis*) to 100.0% (six organisms). Only three strains of Enterobacteriaceae had a tigecycline MIC at >4 mg/L (resistant).
- Among Gram-positive species with tigecycline breakpoints approved by the USA-FDA, all streptococci had MIC values at ≤0.25 mg/L (susceptible). Only three MRSA had a tigecycline MIC at 1 mg/L, only one log_2 dilution step above the breakpoint. All enterococci (including 128 *E. faecalis* and 36 *E. faecium*) were susceptible to tigecycline (MIC_{90}, 0.25 mg/L).
- Several noteworthy resistance patterns were identified (Tables 2 and 3).
  - *E. cloacae* (3 isolates) with elevated carbapenem MIC results having IMP-1 (2) or IMP-21 (1) metallo-ß-lactamases.
  - *P. mirabilis* isolates producing CTX-M-2 from nine hospitals and showing clonal spread.
  - *S. agalactiae* (13 hospitals) with fluoroquinolone resistance; QRDR mutations (two per isolate) were noted (Table 2).
  - *S. pneumoniae* with MDR patterns, some showing greater resistance to extended spectrum cephalosporins compared to penicillin G.
  - *Acinetobacter* spp. were usually susceptible to most agents but two strains produced one or more carbapenemases (OXA-58 + IMP-1, *A. lwoffii*; OXA-23).
- *P. aeruginosa* continues to harbor metallo-ß-lactamases (IMP-1, IMP-7, VIM-2) with evidence of intra- and inter-center spread (Table 3).

Table 1. Tigecycline MIC distributions for 3,902 organisms isolated in 19 medical centers in Japan during 2007.

	Occurrences at MIC (mg/L)									%
Organism (no. tested)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	>4	susceptible
E. coli (199)	0	27	117	51	4	0	0	0	0	100.0
Klebsiella spp. (199)	0	1	17	128	42	3	6	2	0	99.0
Enterobacter spp. (197)	0	0	7	130	44	9	7	0	0	100.0
P. mirabilis (153)	0	0	0	1	14	38	69	29	2	79.7
Serratia spp. (102)	0	0	0	9	48	39	3	2	1	97.1
Acinetobacter spp. (193)	0	15	77	67	14	17	1	2	0	99.0[a]
P. aeruginosa (598)	0	0	0	2	9	21	78	268	220	-
Other Gram-negative (6)	0	0	2	1	0	1	1	0	1	-
S. aureus (1,198)	3	186	503	318	185	3	0	0	0	99.8
Oxacillin-resistant (584)	1	41	180	179	180	3	0	0	0	99.5
Oxacillin-susceptible (614)	2	145	323	139	5	0	0	0	0	100.0
CoNS (198)	7	37	85	58	11	0	0	0	0	-
Enterococci (195)	22	55	62	56	0	0	0	0	0	100.0
S. pneumoniae (459)	401	53	4	1	0	0	0	0	0	-
Penicillin-susceptible (179)	153	22	1	0	0	0	0	0	0	-
Penicillin-intermediate (135)	117	16	2	0	0	0	0	0	0	-
Penicillin-resistant (148)	131	15	1	1	0	0	0	0	0	-
Viridans group streptococci (29)	25	4	0	0	0	0	0	0	0	100.0
ß-haemolytic streptococci (173)	162	5	6	0	0	0	0	0	0	100.0
Other Gram-positive (3)	1	2	0	0	0	0	0	0	0	-

a. USA-FDA breakpoint criteria in tigecycline package insert and those of Jones et al. for Acinetobacter spp. (≤2 mg/L).

Table 2. Comparative tigecycline activity against organisms exhibiting epidemic or endemic occurrences of various resistances to other antimicrobial agents.

		MIC (mg/L)			% by category[a]	
Organism (no.)	Antimicrobial	50%	90%	Range	Susceptible	Resistant
Enterobacter spp. (197)	Tigecycline	0.25	0.5	0.12-2	100.0	0.0
	Minocycline	2	8	0.5->8	89.3	6.1
	Ertapenem	≤0.06	0.5	≤0.06->8	97.5[b]	1.0
	Imipenem	0.5	2	0.25-4	100.0[b]	0.0
	Meropenem	≤0.12	≤0.12	≤0.12-4	100.0[b]	0.0
	Amikacin	2	2	0.5-32	99.5	0.0
	P/T[c]	4	64	≤0.5->64	81.7	5.1
	Levofloxacin	≤0.5	2	≤0.5->4	91.9	5.6
P. mirabilis (153)	Tigecycline	2	4	0.25->4	79.7	1.3
	Minocycline	>8	>8	2->8	2.6	84.3
	Ceftazidime	≤1	≤1	≤1-16	99.3	0.0 (2.0)[d]
	Ceftriaxone	≤0.25	2	≤0.25->32	90.8[e]	9.2 (11.8)[d]
	Ertapenem	≤0.03	≤0.06	≤0.06-0.12	100.0	0.0
	Imipenem	1	2	0.12-8	99.3	0.0
	Meropenem	≤0.12	≤0.12	≤0.12	100.0	0.0
	Amikacin	4	4	0.5-8	100.0	0.0
	P/T	≤0.5	1	≤0.5-2	100.0	0.0
	Levofloxacin	≤0.5	2	≤0.5->4	90.2	7.2
ß-haemolytic-streptococci (173)	Tigecycline	≤0.03	≤0.03	≤0.03-0.12	100.0	-[c]
	Minocycline	≤2	>8	≤2->8	59.0	39.9
	Penicillin	0.03	0.06	≤0.015-0.12	100.0	-
	Ceftriaxone	≤0.25	≤0.25	≤0.25-0.5	100.0	-
	Levofloxacin	1	>4	≤0.5->4	85.5[f]	14.5
	Erythromycin	≤0.25	>2	≤0.25->2	80.9	17.9
	Clindamycin	≤0.25	≤0.25	≤0.25->2	90.2	9.2
	Vancomycin	0.5	0.5	0.25-1	100.0	-
S. pneumoniae (459)	Tigecycline	≤0.03	0.06	≤0.03-0.25	-	-
	Tetracycline	>8	>8	≤2->8	15.3	84.3
	Penicillin	0.25	2	≤0.015-4	38.3	32.2
	Cefepime	0.5	1	≤0.12-8	92.4[g]	2.0
	Ceftriaxone	0.5	2	≤0.25-16	84.3[g]	4.4
	Levofloxacin	1	1	≤0.5->4	98.5	1.5
	Erythromycin	>2	>2	≤0.25->2	13.3	86.3
	Clindamycin	>2	>2	≤0.25->2	41.8	58.0
Acinetobacter spp. (193)	Tigecycline	0.25	1	0.06-4	99.0	0.0
	Minocycline	0.12	1	≤0.06-8	97.9	0.0
	Amikacin	2	8	≤0.25->32	96.9	3.1
	A/S[c]	≤2	8	≤2->16	91.2	7.3
	Cefepime	2	8	0.25->16	90.2	6.7
	Imipenem	0.5	1	≤0.12->8	97.4[h]	1.6
	Levofloxacin	≤0.5	4	≤0.5->4	88.1	9.3
	Polymyxin B	≤0.5	≤0.5	≤0.5-2	100.0	0.0

a. Interpretive category breakpoints of the USA-FDA (tigecycline) or the CLSI.
b. Three strains of *E. cloacae* with IMP-1 (2) or IMP-21 (1); 1.52% overall.
c. P/T = piperacillin/tazobactam; - = no criteria available; A/S = ampicillin/sulbactam.
d. Percentage in parentheses is the ESBL phenotype rate.
e. 14 strains having an ESBL (CTX-M-2).
f. Fluoroquinolone-resistant group B streptococci from 13 hospitals (1-6 per center), all with *gyrA* S83C and *parC* S79F or Y mutations of QRDR.
g. Subset of penicillin-susceptible and cefepime and/or ceftriaxone-non-susceptible isolates.
h. Two strains with carbapenemases (OXA-58 + IMP-1 and OXA-23).

Table 3. Occurrences of metallo-ß-lactamase-producing *P. aeruginosa* by medical center and evaluations of clonal occurrences.

Medical center code	Enzyme type (no. strains)	PFGE patterns (no.)
505	IMP-7 (3)	A (3)
508	IMP-7 (1)	A (1)
	IMP-1 (1)	B (1)
511	IMP-1 (2)	C (2)
	VIM-2 (2)	G (2)
513	IMP-1 (4)	D (2)
		E (1)
		F (1)

CONCLUSIONS

- Tigecycline exhibited excellent activity against contemporary (2007) Japanese isolates (except *P. aeruginosa*) similar to that observed for a 2003-2004 sample.
  - 100.0% susceptibility for *E. coli*, *Enterobacter* spp., MSSA, enterococci, viridans group and ß-haemolytic streptococci (1,407 strains).
  - ≥99.0% susceptibility for *Klebsiella* spp., *Acinetobacter* spp. and all *S. aureus* (1,595 strains).
  - Only seven (7) strains were either non-susceptible or resistant.
- Tigecycline possesses a spectrum and potency active against many clinical isolates in Japan, including MDR strains. Further studies should be considered.