Tigecycline In vitro Susceptibility Test Accuracy Using Three Methods and USA-FDA Interpretive Criteria When Testing Japanese Clinical Isolates (2007)

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RN JONES, K YANAGIHARA, GJ MOET, PR RHOMBERG, S KOHNO
JMI Laboratories, North Liberty, Iowa, USA; Nagasaki University, Nagasaki, Japan

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JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

ABSTRACT

Background: Susceptibility testing accuracy is critical to individual patient care, local epidemiology and surveillance of emerging resistance. Tigecycline (TGC), a novel "first in class" glycylcycline, was tested by three methods (reference broth microdilution [BMD], Etest [AB BIODISK] and disk diffusion [DD]) using nearly 1,000 Japanese clinical strains isolated in 2007.

Methods: 999 strains included *Acinetobacter* spp. (ACB; 100), Enterobacteriaceae (ENT; 400), *S. aureus* (SA; 200), enterococci (ETC; 100), *S. pneumoniae* (SPN; 100) and other streptococci (99), each concurrently tested by CLSI BMD, DD and Etest. Interpretive criteria were those of the USA-FDA product package insert, or proposed by Jones et al. (2007) for ACB. Gentamicin (GEN), minocycline (MIN) and tetracycline (TET) were used as controls and for intermethod accuracy comparisons.

Results: When testing strains from Japan, the categorical concordance (CC) between BMD and DD by drug was (CC/very major error): TGC (98.5/0.0%), GEN (98.2/0.4), MIN (92.8/0.8) and TET (95.2/0.9). Trends toward elevated minor errors (DD false-intermediate using ≥19 mm) were noted for TCG versus ENT and ACB. Etest produced higher TGC MIC results for all organisms except SA (Table; +0.6 x $\log_2$).

Organisms (no. tested)	Log₂ variation of Etest value: >-2	-2	-1	0	+1	+2	>+2
ACB (100)	0	0	24	21	48[a]	7	0
ENT (400)	0	1	33	63	248	55	0
SA (200)	0	20	105	46	29	0	0
ETC (100)	0	9	29	18	32	9	3
SPN (100)	0	2	16	24	57	1	0
Other strept (99)	0	0	14	36	46	3	0
All (999)	0	32	221	208	406	75	3

a. Underlined value represents mode of all values per organism or overall.

Conclusions: TGC susceptibility testing using USA-FDA or other proposed interpretive criteria and CLSI BMD or DD methods appears acceptable for use in Japan (999 isolates screened). Etest MIC results, however, are slightly elevated compared to CLSI BMD and could produce false-non-susceptible TGC results; also USA-FDA DD breakpoints applied to ENT should require a slight modification.

INTRODUCTION

Tigecycline, released by the United States (USA) FDA in 2005, was the first glycylcycline used in clinical practice on a global level. This semisynthetic derivative of minocycline has a unique, generally bacteriostatic effect that binds to bacterial ribosomes in a manner preventing the two principal determinants of tetracycline resistances. Cross- or co-resistance of tigecycline with other antimicrobial classes among Gram-positive cocci, Enterobacteriaceae and *Acinetobacter* spp. is very rare; therefore, potential applications to the treatment of multidrug-resistant (MDR) species occurring in Japan may produce better clinical outcomes.

A component of a large collection of contemporary (2007) Gram-positive and -negative isolates from 19 hospitals in Japan was selected for detailed in vitro studies. The testing of these representative species strains focused on those organisms of greatest concern regarding emerging antimicrobial resistances in Japan and worldwide (Infectious Disease Society of America). A central laboratory design (JMI Laboratories, North Liberty, Iowa USA) was employed to assure data and procedural accuracy via use of rigid quality control (QC), concurrent reviews by medical directors and interpretations of results by medical technologists having decades of experience. Testing of tigecycline was emphasized by application of the reference Clinical and Laboratory Standards Institute (CLSI; broth microdilution and disk diffusion) methods, and comparisons to Etest results (AB BIODISK, Solna, Sweden).

MATERIALS AND METHODS

Bacterial isolates: A total of 999 bacterial isolates collected during 2007 in 19 medical centers located in Japan were evaluated as part of the Japanese component for 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 requiring minimal numbers by species or genus. 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 reference tested using the CLSI M7-A7 (2006) method. Fresh cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Categorical interpretations for comparison drugs were those found in CLSI M100-S18 (2008); breakpoints for tigecycline were those of the USA-FDA product package insert (2005) or proposed by Jones et al. (2007). QC and procedural assurances were performed using *E. coli* ATCC 25922, *S. aureus* ATCC 29213 and 25923 and *P. aeruginosa* ATCC 27853; all QC results were found within ranges published in CLSI M100-S18 (2008).

Disk diffusion tests were also performed using CLSI M2-A9 (2006) methods. Etest results were generated concurrently by manufacturer recommended procedures (AB BIODISK package inserts) for organisms in six groups (*Acinetobacter* spp., Enterobacteriaceae, *S. aureus*, *S. pneumoniae*, ß-haemolytic streptococci and viridans group streptococcal species). Analyses used guidelines listed in CLSI M23-A2 (2001).

Three control agents were tested by all three methods e.g. gentamicin (700 strains), minocycline (500 strains) and tetracycline (999 strains). Only organism groups having CLSI (2008) interpretive criteria for the comparator and control agents were analyzed.

ACKNOWLEDGEMENT

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RESULTS

- Across all studied Japanese organism groups, tigecycline demonstrated potent activity (MIC_{90}, ≤0.03 to 1 µg/ml) and susceptibility rates varying from 99.3 (Enterobacteriaceae) to 100.0% (all other studied groups), see Table 1.

Table 1. Tigecycline activity tested against 999 contemporary (2007) pathogens isolated from clinical infections in Japan.

Organisms (no. tested)	MIC (µg/ml):[a] 50%	90%	Range	% susceptible[b]
Acinetobacter spp. (100)	0.25	0.5	0.06-1	100.0
Enterobacteriaceae (400)	0.25	1	0.06->4	99.3
S. aureus (200)	0.12	0.5	0.06-0.5	100.0
Enterococci (100)	0.12	0.25	≤0.03-0.25	100.0
S. pneumoniae (100)	≤0.03	0.06	≤0.03-0.25	NA[C]
Other streptococci (99)	≤0.03	≤0.03	≤0.03-0.06	100.0

a. MIC values generated by reference CLSI broth microdilution method M7-A7 (2006).
b. USA-FDA tigecycline package insert or per Jones et al. (2007) for *Acinetobacter* spp.
c. NA = not applicable.

Figure 1. Scattergram comparing reference tigecycline MIC and disk diffusion zone diameters for 999 strains from Japan. Vertical lines indicate USA-FDA breakpoints for Enterobacteriaceae & horizontal lines the MIC breakpoints for Enterobacteriaceae. All errors were found among *A. baumannii* & Enterobacteriaceae (15; 0.9% minor errors). Vertical broken lines are proposed modifications by Jones et al. (2007).

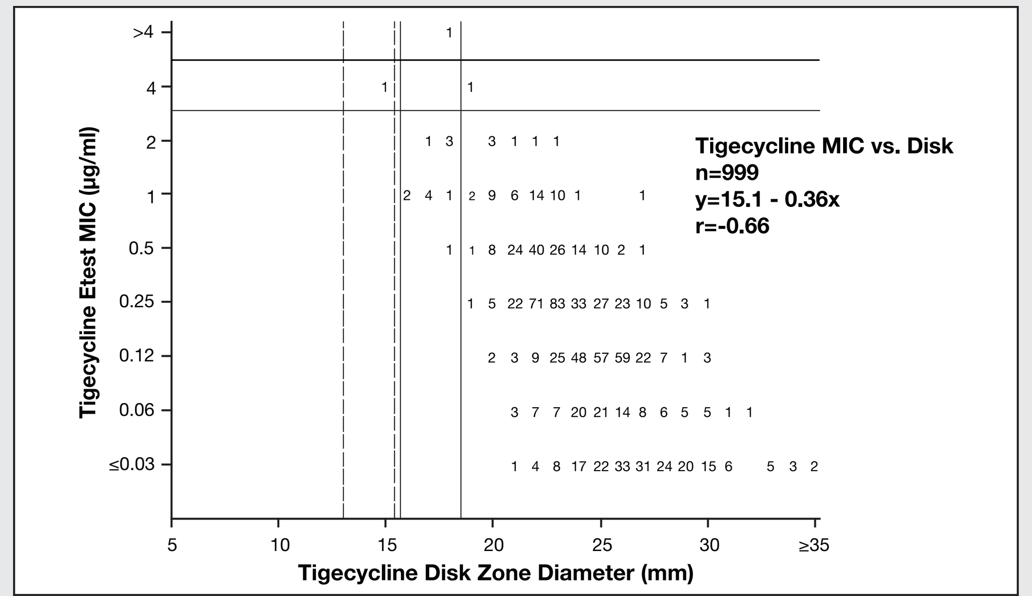


Table 2. Calculated accuracy and interpretive error rates comparing the tigecycline and control drug disk diffusion test results to the CLSI reference broth microdilution method (999 strains from Japan; 2007).

Antimicrobial/organism group (no. tested)	Error (%): Very Major	Major	Minor	% absolute categorical agreement
Tigecycline				
Acinetobacter spp. (100)[a]	0.0	0.0	0.0	100.0
Enterobacteriaceae (400)[b]	0.0 (0.3)	0.0 (0.3)	2.3 (0.0)	97.7 (99.4)
S. aureus (200)[b]	0.0	0.0	0.0	100.0
Enterococci (100)[b]	0.0	0.0	0.0	100.0
S. pneumoniae (100)	NA[c]	NA	NA	NA
Other streptococci (99)[b]	0.0	0.0	0.0	100.0
All strains (999)	0.0 (0.1)	0.0 (0.1)	0.9 (0.0)	99.1 (99.8)
Gentamicin				
All strains (700)[d]	0.4	0.0	1.4	98.2
Minocycline				
All strains (500)[d]	0.8	0.0	6.4	92.8
Tetracycline				
All strains (999)[d]	0.9	0.0	3.9	95.2

a. Breakpoint criteria proposed by Jones et al., 2007; susceptible at ≥16 mm.
b. Tigecycline USA-FDA package insert criteria; Jones et al. (2007) criteria rates in parenthesis, if different. CLSI (2008) interpretative criteria for species, where published.
c. NA = not applicable.
d. CLSI (2008) interpretative criteria for species, where published.

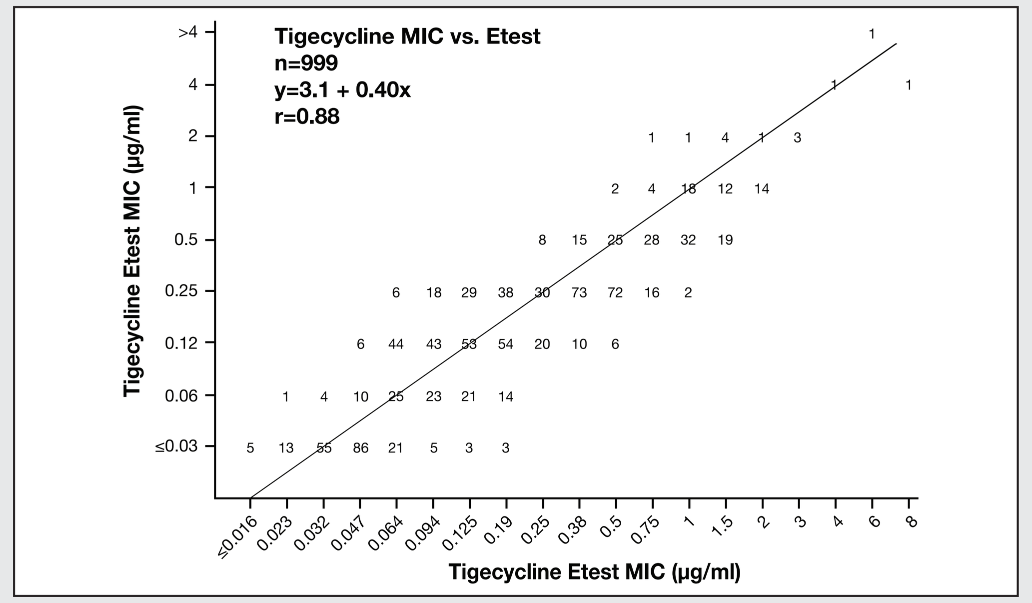
Table 3. Comparisons of Etest MIC for tigecycline with those generated by CLSI reference broth microdilution method (999 strains from Japan; 2007).

Organism group (no. tested)	Log₂ variation of the Etest MIC: -2	-1	0	+1	+2	+3
Acinetobacter spp. (100)	0	24	21	48[a]	7	0
Enterobacteriaceae (400)	1	33	63	248	55	0
S. aureus (200)	20	105	46	29	0	0
Enterococci (100)	9	29	18	32	9	3
S. pneumoniae (100)	2	16	24	57	1	0
Other streptococci (99)[b]	0	14	36	46	3	0
All (999)	32	221	208	406	75	3

a. Underlined value represents mode of all values.
b. Includes ß-haemolytic and viridans group species.

- Intermethod comparisons of CLSI reference broth microdilution and disk diffusion tigecycline results, interpreted by USA-FDA breakpoints, showed excellent categorical agreement (CA; 99.1%), regardless of organism group tested (Table 2). Proposed modifications of breakpoint criteria by Jones et al. (2007) for *Acinetobacter* spp. or Enterobacteriaceae, enhanced the intermethod CA to 99.9%. Comparison drug absolute CA was 92.8-98.2% with acceptable levels of false-susceptible (very major) errors at 0.4-0.9%.
- Figure 1 illustrates a trend toward false-intermediate disk diffusion results among the tested Enterobacteriaceae strains.

Figure 2. Comparison scattergram of Etest tigecycline results (x-axis) and the reference CLSI broth microdilution MICs (999 strains from Japan; 2007).



- Table 3 shows that the Etest results for tigecycline were routinely skewed toward a two-fold higher value for all organism groups except *S. aureus*, where the modal MIC was two-fold lower than CLSI reference broth microdilution results. Overall, the Etest MIC for tigecycline would be expected to be approximately 0.5X one $\log_2$ dilution step elevated as read by technologists experienced with Etest endpoint reading. Essential agreement ± one $\log_2$ dilution was 89.0%.
- Figure 2 shows the Etest/reference MIC comparisons of tigecycline results. The tigecycline MIC values generated by Etest trend toward higher results.

CONCLUSIONS

- Tigecycline demonstrated broad-spectrum activity against a collection of recent (2007) clinical isolates from 19 different medical centers in Japan (99.3-100.0% susceptibility), indicating possible clinical value in this nation.
- Intermethod categorical agreement between tigecycline CLSI reference MIC method results and those generated by the CLSI disk diffusion or Etest methods were acceptable. However, possible modifications of USA-FDA disk diffusion interpretive criteria (*Acinetobacter* spp. and Enterobacteriaceae) could improve CA.
- Etest MIC results were elevated overall and could produce false-non-susceptible values for species having MIC values near existing or proposed breakpoints e.g. *Acinetobacter* spp. and Enterobacteriaceae.