

Comparative In Vitro Antimicrobial Activity of Tigecycline, a New Glycylcycline Compound, in Freshly Prepared Medium and Quality Control[∇]

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The in vitro spectra of activity of tigecycline and tetracycline were determined for 2,490 bacterial isolates representing 50 different species or phenotypic groups. All isolates were tested simultaneously by broth microdilution using freshly prepared Mueller-Hinton broth and by disk diffusion. Portions of these data were submitted to the Food and Drug Administration (FDA) in support of the sponsor's application for new drug approval. In a separate study, MIC and disk diffusion quality control ranges were determined. The tigecycline MICs at which 50%/90% of bacteria were inhibited were (in µg/ml) as follows: for *Streptococcus* spp., 0.06/0.12; for *Moraxella catarrhalis*, 0.06/0.12; for *Staphylococcus* spp., 0.12/0.25; for *Enterococcus* spp., 0.12/0.25; for *Listeria monocytogenes*, 0.12/0.12; for *Neisseria meningitidis*, 0.12/0.25; for *Haemophilus* spp., 0.25/0.5; for *Enterobacteriaceae*, 0.05/2.0; for non-*Enterobacteriaceae*, 0.5/8.0. Tigecycline was consistently more potent than tetracycline against all species studied. The data from this study confirm the FDA-approved MIC and disk diffusion breakpoints for tigecycline for *Streptococcus* spp. other than *Streptococcus pneumoniae*, enterococci, and *Enterobacteriaceae*. Provisional breakpoints for *Haemophilus* spp. and *S. pneumoniae* are proposed based on the data from this study. The following MIC and/or disk diffusion quality control ranges are proposed: *Staphylococcus aureus* ATCC 29213, 0.03 to 0.25 µg/ml; *S. aureus* ATCC 25923, 20 to 25 mm; *Escherichia coli* ATCC 25922, 0.03 to 0.25 µg/ml and 20 to 27 mm; *Pseudomonas aeruginosa* ATCC 27853, 9 to 13 mm, *Enterococcus faecalis* ATCC 29212, 0.03 to 0.12 µg/ml; *S. pneumoniae* ATCC 49619, 0.015 to 0.12 µg/ml and 23 to 29 mm; *Haemophilus influenzae* ATCC 49247, 0.06 to 0.5 µg/ml and 23 to 31 mm; and *Neisseria gonorrhoeae* ATCC 49226, 30 to 40 mm.

Tigecycline (formerly GAR-936) is a new glycylcycline (24) compound with a broad spectrum of antibacterial activity (2–4, 8, 10, 13, 14, 23). In addition, it has been shown to be active against microorganisms known to be resistant to other classes of antimicrobial agents (1, 11, 12, 19, 20, 22). Recent studies have found that the age of the broth medium used for in vitro susceptibility testing can affect the MICs of tigecycline by as much as two to eightfold (5, 21). Susceptibility tests performed with medium which was ≤12 h old at the time of testing produced MICs which were substantially lower than those observed with medium that had been aged for various periods of time up to 1 month. The effects of medium aging could be counteracted by the addition of Oxyrase, a biocatalytic oxygen-reducing reagent, to the susceptibility testing medium. Further testing revealed that dissolving tigecycline in aged medium resulted in the formation of an oxidized tigecycline product which had decreased antibacterial activity (5). Once the MIC trays were frozen, the oxidation of the drug was minimal. MIC trays which were prepared using freshly prepared medium and stored at –20°C for several weeks produced results which were nearly identical to those obtained with trays tested on the same day as production with freshly prepared broth medium. These observations have resulted in the Clinical and Laboratory Standards Institute (CLSI) requirement that only freshly prepared

medium (≤12 h old) be used for testing tigecycline by broth MIC methods (7).

Unfortunately, spectrum-of-activity studies published prior to this may be flawed in the respect that they were performed using agar dilution or using broth medium in which the age of the medium was either unknown or unstated.

Tigecycline is currently approved worldwide for the treatment of serious skin, skin structure, and intra-abdominal bacterial infections in hospitals. Bacterial isolates obtained during the clinical trials were tested and reported using freshly prepared medium which was less than 12 h old at the time of MIC tray production (6).

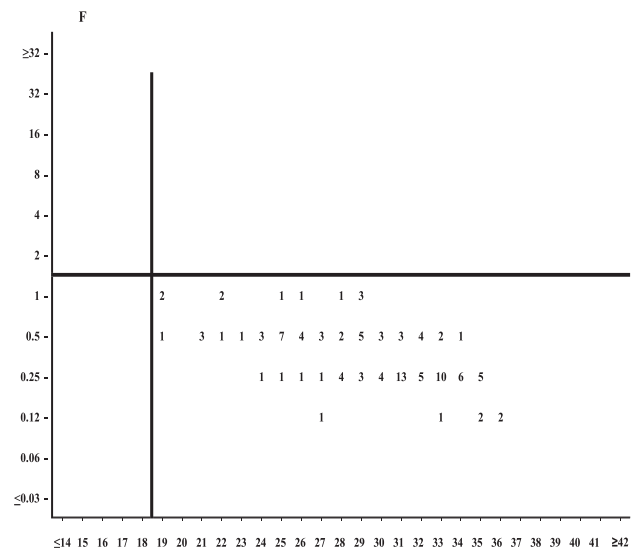
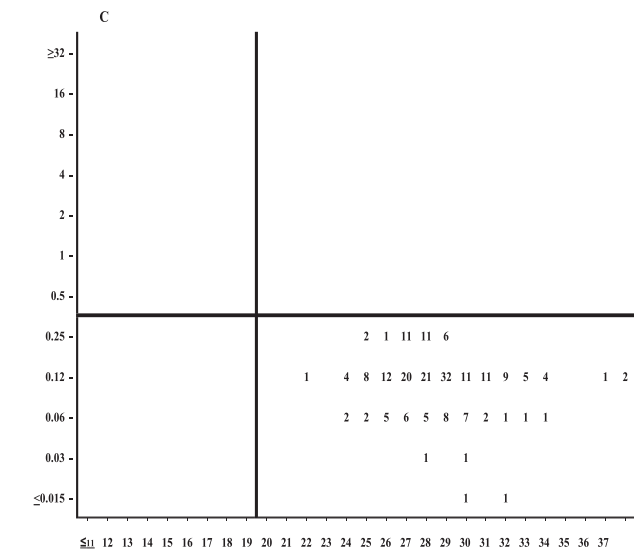
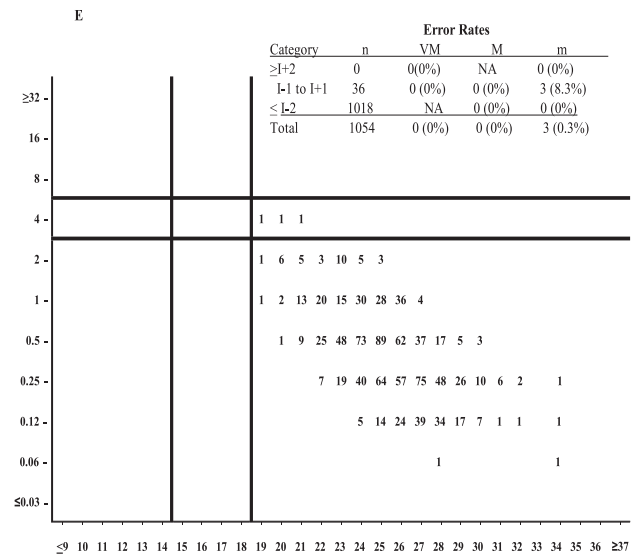
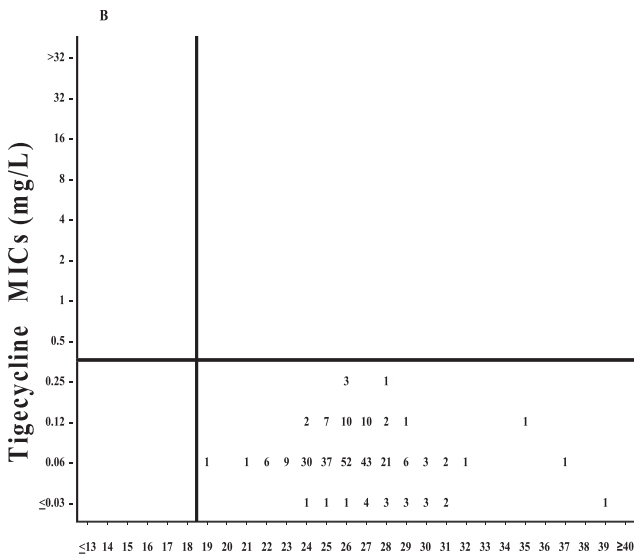
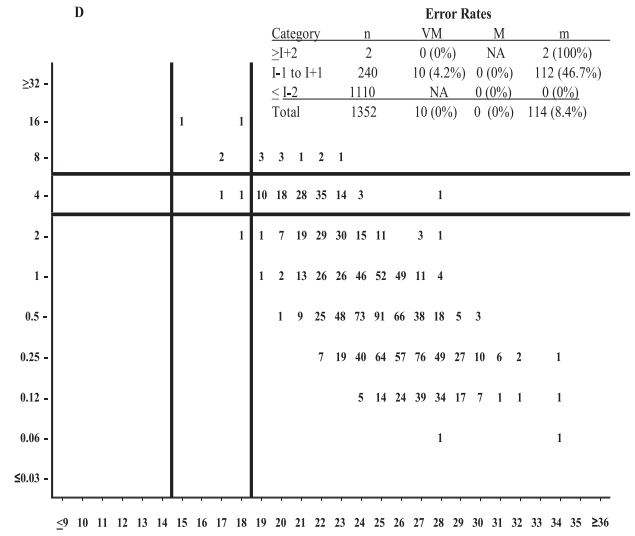
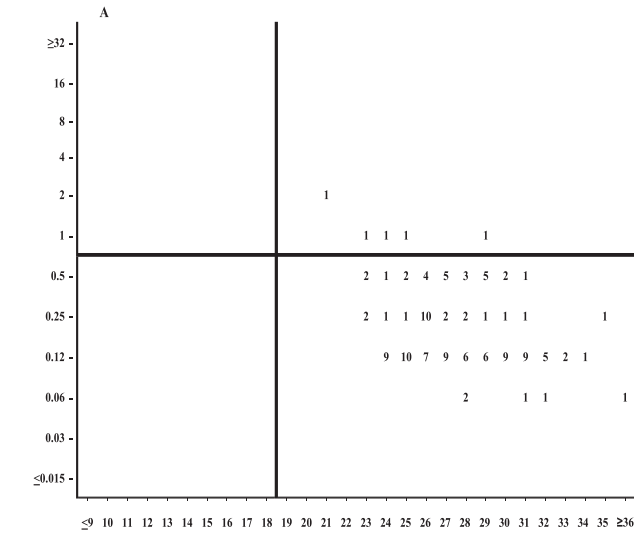
The present study was designed to compare the in vitro antibacterial activity of tigecycline with that of tetracycline against a broad range of bacterial pathogens, provide supplemental data for the sponsor's FDA new-drug application, determine correlation of tigecycline disk diffusion to MIC results for these microorganisms, and propose MIC and disk diffusion quality control ranges for eight different aerobic quality control strains.

MATERIALS AND METHODS

Bacteria tested. A total of 2,490 recent clinical bacterial isolates were selected as representative pathogens that cause infections for which tigecycline might be considered for therapy. All isolates were obtained from medical centers across North America and sent to the Clinical Microbiology Institute, where they were reidentified and placed in a large culture collection. These included 269 streptococci, 216 enterococci, 44 *Staphylococcus aureus* isolates, 86 coagulase-negative staphylococci, 1,352 *Enterobacteriaceae*, 153 nonfermentative gram-negative rods, 100 *Listeria monocytogenes* isolates, 64 *Moraxella catarrhalis* isolates, 93

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Tigecycline 15 μ g Disc Zone Diameter (mm)

TABLE 1. Sites participating in quality control studies

Participant	Institution, city, and state or province
S. Brown.....	Clinical Microbiology Institute, Wilsonville, OR
M. Bauman ^a	St. Vincent's Hospital, Portland, OR
M. Cox ^b	Anaerobe Systems, Morgan Hill, CA
M. J. Ferraro ^a	Massachusetts General Hospital, Boston, MA
D. Hardy.....	Univ. of Rochester Medical Center, Rochester, NY ^d
J. Hindler.....	UCLA Medical Center, Los Angeles, CA
S. Jenkins ^a	Carolinas Medical Center, Charlotte, NC
C. Knapp.....	TREK Diagnostic Systems, Westlake, OH
G. Overturf ^c	Univ. New Mexico Medical Center, Albuquerque, NM
G. Procop.....	Cleveland Clinic, Cleveland, OH
R. Rennie.....	Univ. of Alberta Hospital, Edmonton, Alberta, Canada
M. Saubolle ^a	Good Samaritan Hospital, Phoenix, AZ
E. J. Baron ^c	Stanford Univ. Hospital and Clinics, Stanford, CA
F. Tenover ^c	Centers for Disease Control and Prevention, Atlanta, GA

^a *S. aureus*, *E. coli*, and *E. faecalis* MIC study only.
^b Disk diffusion study only.
^c *S. pneumoniae* and *H. influenzae* MIC study only.
^d Univ., university.

Neisseria meningitidis isolates, and 113 *Haemophilus* spp. Two of the goals of this study were to test approximately 100 strains of species identified as a potential target for tigecycline therapy and to provide the sponsor with supplemental data for *Staphylococcus* spp. This collection includes isolates with a variety of previously established resistance phenotypes.

Antimicrobial susceptibility testing. Tigecycline was provided as a sterile powder (lot no. MB1611) by Wyeth Research (Pearl River, NY). Tetracycline (lot no. 092K1607) was purchased from Sigma (St. Louis, MO) and was used as an internal quality control. The results for tigecycline were compared to those for tetracycline as a method of establishing relative potency. Disk diffusion susceptibility tests used commercially prepared 15-µg disks of tigecycline (lot no. 3259023) and 30-µg disks of tetracycline (lot no. 0175726) purchased from BD Microbiologic Systems (Cockeysville, MD).

All aerobic organisms were tested by the broth microdilution method recommended by the CLSI (15) using cation-adjusted Mueller-Hinton broth which was less than 12 h old at the time of MIC tray production. The medium was supplemented with 3% lysed horse blood for testing of all streptococci, *L. monocytogenes*, and *N. meningitidis* or made up as *Haemophilus* test medium for testing of *Haemophilus* spp. as recommended by the CLSI. All organisms were tested simultaneously by the disk diffusion method outlined by the CLSI (16) using Mueller-Hinton agar plus 5% sheep blood (streptococci, *L. monocytogenes*, *M. catarrhalis*, and *N. meningitidis*), *Haemophilus* test medium agar (*Haemophilus* spp.), or plain Muller-Hinton agar (all other genera).

MIC-versus-zone-diameter scattergrams (Fig. 1A to F) were prepared using an error minimization approach (17). FDA disk diffusion interpretive criteria are confirmed or proposed for all strains.

Quality control studies. Three separate multilaboratory studies were undertaken in order to propose quality control ranges for MIC and disk diffusion methodologies. The testing laboratories included both hospital and commercial microbiology laboratories in the United States and are identified in Table 1. These studies closely followed the protocol described by the CLSI (17) with the exception that the number of testing facilities exceeded the minimum number of seven required sites. In addition, a total of up to six lots of broth medium was tested rather than the minimum of three lots. The quality control organisms were those recommended by the CLSI (18) and included *S. aureus* ATCC 29213 (MIC only) and ATCC 25923 (disk only), *Enterococcus faecalis* ATCC 29212 (MIC only), *Streptococcus pneumoniae* ATCC 49619 (MIC and disk), *Pseudomonas aeruginosa* ATCC 27853 (disk only), *Escherichia coli* ATCC 25922 (MIC and

TABLE 2. FDA-approved susceptibility test result interpretive criteria for tigecycline

Pathogen	MIC (µg/ml) ^a			Disk diffusion (zone diam, mm)		
	S	I	R	S	I	R
<i>S. aureus</i>	≤0.5			≥19		
<i>Streptococcus</i> spp. other than <i>S. pneumoniae</i>	≤0.25			≥19		
<i>E. faecalis</i> (vancomycin susceptible only)	≤0.25			≥19		
<i>Enterobacteriaceae</i> ^b	≤2	4	≥8	≥19	15–18	≤14

^a The current absence of resistant isolates precludes defining any results other than “susceptible.” Isolates yielding MIC results suggestive of the “nonsusceptible” category should be submitted to a reference laboratory for further testing.
^b Tigecycline has decreased in vitro activity against *Morganella* spp., *Proteus* spp., and *Providencia* spp.

disk), *Neisseria gonorrhoeae* ATCC 49226 (disk only), and *Haemophilus influenzae* ATCC 49247 (MIC and disk). Internal quality control results for the control drug, tetracycline, were within published ranges available (18) for the majority of tests. When any control value was out of the established ranges, all of the tigecycline data associated with that day's testing were discarded. This study involved 10 replicate tests on up to six lots of Mueller-Hinton broth or three lots of agar, two lots of 15-µg tigecycline disks (BDMS lot no. 0167721 and Oxoid lot no. 241765), and one lot of 30-µg tetracycline disks (BDMS lot no. 1050730). This exercise generated a target of 600 MICs and 480 disk diffusion zone diameters with each appropriate quality control strain. Zone diameters were evaluated using the statistics of Gavan et al. (9).

RESULTS

Spectrum-of-activity and interpretive-criteria study. Table 3 summarizes the MICs of tigecycline and tetracycline against all bacterial isolates tested. Tigecycline exhibited excellent activity against all gram-positive species, *M. catarrhalis*, *H. influenzae*, and *N. meningitidis*, with MICs at which 90% of bacteria were inhibited (MIC_{90s}) of ≤0.5 µg/ml. For the majority of these species, tigecycline was 2 to 1,024 times more potent than tetracycline. MICs were not significantly affected by increasing resistance to penicillin (*S. pneumoniae*), oxacillin (staphylococci), vancomycin (enterococci), or the presence of extended-spectrum β-lactamases (ESBLs) (*E. coli* and *Klebsiella pneumoniae*). In each of these instances, the MIC₉₀ of the resistant strains was within 1 doubling dilution of that of the more susceptible isolates. Good activity was also noted for *Haemophilus parainfluenzae* and the majority of the *Enterobacteriaceae* species. The MIC_{90s} for these species ranged from 0.25 µg/ml (*Citrobacter koseri*) to 2 µg/ml (*Klebsiella oxytoca*, ESBL⁺ *K. pneumoniae*, *Proteus vulgaris*, *Serratia marcescens*, and *Salmonella enterica* serovar Typhimurium). Good activity was also noted for *Pasteurella multocida* (MIC₉₀ = 0.25 µg/ml). Decreased in vitro activity was noted for *Morganella* spp., *Pro-*

FIG. 1. Scattergrams of tigecycline MICs versus zone diameters (15-µg disks). (A) All staphylococci combined (including methicillin-resistant isolates; n = 130), (B) *Streptococcus pneumoniae* (n = 209) and nonpneumococcal streptococci (n = 60) combined, (C) all *Enterococcus* spp. combined (n = 216), (D) all *Enterobacteriaceae* combined (n = 1,352) (122/125 [97.6%] of the isolates with a tigecycline MIC of ≥4 µg/ml were *Morganella*, *Proteus* spp. other than *P. vulgaris*, or *Providencia* spp.), (E) *Enterobacteriaceae* without *Morganella*, *Proteus* spp. other than *P. vulgaris*, or *Providencia* spp. (n = 1,054), (F) *Haemophilus* spp. (n = 113). Horizontal lines represent proposed susceptible (lower line) and resistant (upper line) MIC breakpoints; vertical lines represent proposed susceptible (right line) and resistant (left line) zone diameter breakpoints. Abbreviations: n, number of strains tested; VM, very major errors; M, major errors; m, minor errors; I+2, intermediate MIC breakpoint plus 2 log₂ dilutions; I-1 to I+1, intermediate MIC breakpoint plus or minus 1 log₂ dilution; I-2, intermediate MIC breakpoint minus 2 log₂ dilutions.

TABLE 3. Susceptibilities of aerobic bacteria to tigecycline and tetracycline

Species (<i>n</i> ^a) ^b	MIC of drug (μg/ml)					
	Tigecycline			Tetracycline		
	Range	50%	90%	Range	50%	90%
All strains combined (2,490)	0.015–32	0.25	2	0.015–>32	2	>32
Gram-positive strains						
All streptococci combined (269)	0.03–0.25	0.06	0.12	0.12–>32	0.5	>32
<i>S. agalactiae</i> (20)	0.06–0.06	0.06	0.06	0.25–>32	32	>32
<i>S. pneumoniae</i> , penicillin-S (23)	0.03–0.12	0.06	0.06	0.12–0.5	0.25	0.5
<i>S. pneumoniae</i> , penicillin-I (100)	0.03–0.25	0.06	0.12	0.12–>32	0.25	>32
<i>S. pneumoniae</i> , penicillin-R (86)	0.03–0.25	0.06	0.12	0.25–>32	32	32
<i>S. pyogenes</i> (40)	0.03–0.06	0.06	0.06	0.12–32	0.25	16
All staphylococci combined (130)	0.06–2	0.12	0.25	0.12–>32	0.25	>32
All <i>S. aureus</i> strains combined (44)	0.12–1	0.12	0.5	0.25–>32	0.25	>32
<i>S. aureus</i> , methicillin-R (24)	0.12–1	0.12	0.25	0.25–>32	0.25	>32
<i>S. aureus</i> , methicillin-S (20)	0.12–0.25	0.12	0.12	0.25–>32	0.25	0.5
All coagulase-negative staphylococci combined (86)	0.06–2	0.12	0.5	0.12–>32	1	>32
<i>S. epidermidis</i> , methicillin-R (21)	0.06–0.5	0.12	0.5	0.25–>32	2	32
<i>S. epidermidis</i> , methicillin-S (35)	0.06–1	0.12	0.5	0.12–>32	1	>32
<i>S. haemolyticus</i> (30)	0.06–2	0.25	0.5	0.12–>32	0.5	>32
All enterococci combined (216)	0.015–0.25	0.12	0.25	0.015–>32	2	>32
<i>E. avium</i> (40)	0.015–0.25	0.12	0.12	0.015–>32	16	>32
<i>E. casseliflavus</i> (23)	0.12–0.25	0.12	0.25	0.25–32	1	32
<i>E. faecalis</i> , vancomycin-R (34)	0.06–25	0.12	0.25	0.25–>32	1	>32
<i>E. faecalis</i> , vancomycin-S (19)	0.12–25	0.12	0.25	1–>32	32	>32
<i>E. faecium</i> , vancomycin-R (20)	0.06–0.25	0.12	0.12	0.25–>32	16	>32
<i>E. faecium</i> , vancomycin-S (40)	0.06–0.25	0.12	0.12	0.12–>32	0.5	>32
<i>E. gallinarium</i> (40)	0.06–0.25	0.12	0.25	0.25–>32	1	>32
<i>Listeria monocytogenes</i> (100)	0.06–0.12	0.12	0.12	0.25–>32	1	1
Gram-negative strains						
<i>Moraxella catarrhalis</i> (64)	0.06–0.25	0.06	0.12	0.06–16	0.25	0.5
<i>Neisseria meningitidis</i> (93)	0.03–0.5	0.12	0.25	0.03–1	0.25	0.5
All <i>Haemophilus</i> spp. combined (113)	0.12–1	0.25	0.5	0.25–>32	0.5	2
<i>H. influenzae</i> (64)	0.12–1	0.25	0.5	0.25–32	0.5	0.5
<i>H. parainfluenzae</i> (49)	0.12–1	0.5	1	0.25–>32	0.5	8
All <i>Enterobacteriaceae</i> combined (1,352)	0.06–16	0.5	2	0.25–>32	16	>32
<i>Citrobacter freundii</i> (20)	0.12–2	0.25	0.5	0.5–>32	1	2
<i>Citrobacter koseri</i> (41)	0.12–1	0.25	0.25	1–4	1	1
<i>Enterobacter aerogenes</i> (21)	0.25–1	0.5	1	1–>32	2	>32
<i>Enterobacter cloacae</i> (20)	0.25–0.5	0.5	0.5	1–2	2	2
<i>Escherichia coli</i> (20)	0.12–0.5	0.25	0.5	1–>32	2	>32
<i>Escherichia coli</i> , ESBL ⁺ (20)	0.25–1	0.25	0.5	1–>32	>32	>32
<i>Klebsiella oxytoca</i> (19)	0.25–2	0.5	2	1–8	1	4
<i>Klebsiella pneumoniae</i> (20)	0.5–2	0.5	1	1–8	2	4
<i>Klebsiella pneumoniae</i> , ESBL ⁺ (36)	0.25–4	1	2	1–>32	>32	>32
<i>Morganella morganii</i> (20)	1–8	4	4	2–>32	>32	>32
<i>Proteus mirabilis</i> (20)	1–4	2	4	32–>32	>32	>32
<i>Providencia rettgeri</i> (100)	0.25–16	4	4	1–>32	32	>32
<i>Providencia stuartii</i> (98)	0.25–8	2	4	2–>32	>32	>32
<i>Proteus vulgaris</i> (60)	0.5–2	1	2	1–>32	8	32
<i>Shigella boydii</i> (106)	0.12–1	0.25	0.5	0.25–>32	>32	>32
<i>Shigella dysenteriae</i> (92)	0.12–1	0.25	0.25	1–>32	>32	>32
<i>Shigella flexneri</i> (100)	0.06–1	0.25	0.25	0.5–>32	>32	>32
<i>Shigella sonnei</i> (103)	0.12–1	0.25	0.5	0.5–>32	>32	>32
<i>Salmonella enterica</i>						
Serovar Enteritidis (100)	0.12–2	0.5	1	0.5–>32	1	>32
Serovar Paratyphi (111)	0.12–2	0.5	1	0.5–>32	1	32
Serovar Typhi (105)	0.06–1	0.25	0.5	0.25–>32	1	4
Serovar Typhimurium (100)	0.25–2	1	2	1–>32	>32	>32
<i>Serratia marcescens</i> (20)	1–4	1	2	8–>32	16	>32
All non- <i>Enterobacteriaceae</i> combined (153)	0.06–32	0.5	8	0.12–>32	4	>32
All <i>Acinetobacter</i> spp. (41)	0.12–4	0.5	2	0.25–>32	2	8
<i>Burkholderia cepacia</i> (32)	0.06–16	1	8	0.12–>32	>32	>32
<i>Pseudomonas aeruginosa</i> (20)	8–32	16	16	16–>32	32	>32
<i>Pasteurella multocida</i> (40)	0.06–0.25	0.06	0.25	0.12–32	1	16
<i>Stenotrophomonas maltophilia</i> (20)	0.06–8	1	4	0.25–32	8	32

^a *n*, no. of strains.^b S, sensitive; I, intermediate; R, resistant.

TABLE 4. Tigecycline MIC quality control

Quality control strain	No. of occurrences at MIC (µg/ml) of ^{a,b} :													% in range ^c
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	
<i>E. coli</i> ATCC 25922	0	0	401	176	5	0	0	0	0	0	0	0	0	100
<i>S. aureus</i> ATCC 29213	0	48	194	277	61	1	1	1	0	0	0	0	0	99.5
<i>E. faecalis</i> ATCC 29212	0	86	374	132	5	0	0	0	0	0	0	0	0	99.2
<i>S. pneumoniae</i> ATCC 49619	0	146	90	4	0	0	0	0						100
<i>H. influenzae</i> ATCC 49247	0	0	9	107	113	11	0	0						100

^a The target of 600 replicates per strain was not met for all test species.
^b Numbers within recommended quality control ranges are represented in bold.
^c Percentage of results which fall within the recommended range. Acceptable limit, ≥95%.

teus spp. other than *P. vulgaris*, *Providencia* spp., *Burkholderia cepacia*, *P. aeruginosa*, and *Stenotrophomonas maltophilia*, which had MIC_{90s} of ≥4 µg/ml for tigecycline. A total of 122/125 (97.6%) of the isolates with an individual MIC of ≥4 µg/ml were from among this group of microorganisms.

Figure 1A to F shows the scattergrams of tigecycline MICs versus disk diffusion zone diameters. FDA susceptible-only MIC breakpoints and disk diffusion breakpoints for tigecycline of ≤0.25 µg/ml and ≥19 mm for the nonpneumococcal strep-

tococci and enterococci and ≤0.5 µg/ml and ≥19 mm for the staphylococci and MIC breakpoints of ≤2, 4, and 8 µg/ml and ≥19 mm, 15 to 18 mm, and ≤14 mm for susceptible, intermediate, and resistant for the *Enterobacteriaceae* were confirmed (Table 2). Provisional susceptible-only breakpoints of ≤1 µg/ml and ≥19 mm for *Haemophilus* spp. and ≤0.25 µg/ml and ≥19 mm for *S. pneumoniae* are proposed based on the data from this study. The zone diameter breakpoints proposed were

TABLE 5. Number of occurrences at each zone diameter for tigecycline disk diffusion quality control

Zone diam (mm)	No. of occurrences for strain (% within range) ^a :					
	<i>E. coli</i> ATCC 25922 (99.0)	<i>S. aureus</i> ATCC 25923 (99.1)	<i>P. aeruginosa</i> ATCC 27853 (99.0)	<i>S. pneumoniae</i> ATCC 49619 (99.0)	<i>N. gonorrhoeae</i> ATCC 49226 (99.2)	<i>H. influenzae</i> ATCC 49247 (97.9)
8			1			
9			48			
10			136			
11			206			
12			83			
13			4			
14			2			
15						
16						
17						
18						
19	5	1				
20	10	18				
21	25	47		1		2
22	16	132		2		7
23	58	143		37		9
24	123	78		111		5
25	160	32		104		29
26	70	2		99	2	46
27	11	1		40		69
28	2			48	1	89
29				27	1	81
30				3	10	57
31					24	22
32					41	
33					53	
34					72	
35					93	
36					79	
37					50	
38					34	
39					10	
40					7	
41					1	
42						

^a Numbers within recommended quality control ranges are represented in bold.

designed to minimize the interpretive discrepancies between the two types of susceptibility testing methods.

The error rates for the majority of species were within the limits established by the CLSI (17). The profound activity of tigecycline against all gram-positive isolates is readily apparent. The error rates for *S. pneumoniae* and *Streptococcus* spp. other than *S. pneumoniae* (Fig. 1B), the enterococci (Fig. 1C), and *Haemophilus* spp. (Fig. 1F) were all zero. The staphylococcal isolates (44 *S. aureus* isolates and 86 coagulase negative staphylococci) showed 5 strains above the *S. aureus* FDA sensitive-only breakpoint, including one strain of *S. haemolyticus* which was 2 dilutions above and the 4 strains (3 coagulase-negative staphylococci and a single strain of *S. aureus* known to be methicillin resistant and vancomycin intermediate) which were 1 dilution above the breakpoint (Fig. 1A). This strain of *S. aureus* was retested recently and had a tigecycline MIC of 0.5 µg/ml on retest.

The very major and minor error rates for the *Enterobacteriaceae* (Fig. 1D) were high. The vast majority of these errors were due to *Morganella morganii*, *Proteus* spp. other than *P. vulgaris*, and *Providencia* spp. If these strains were eliminated from consideration, the very-major-error rate would drop to 0 while minor error rates would drop to 3 out of 1,054 isolates or 0.3% (Fig. 1E). Included among these strains are isolates known to produce ESBLs.

Quality control studies. Quality control ranges for MIC testing were proposed on the basis of the modal MICs observed \pm 1 log₂ dilution. If the MIC distribution was bimodal, then a four-dilution range was proposed. Disk diffusion zone diameter ranges were proposed using the method of Gavan et al. (9) with adjustments as needed in order to encompass at least 95% of observed values. The proposed MIC and zone diameter ranges are presented in Tables 4 and 5, respectively. These quality control ranges were accepted by the Antimicrobial Susceptibility Testing Subcommittee of the CLSI at their January 2004 and June 2004 meetings and later by the FDA.

DISCUSSION

The in vitro activity of tigecycline against gram-positive strains, *M. catarrhalis*, *N. meningitidis*, and *H. influenzae*, was excellent. Tigecycline was consistently more potent than tetracycline against all species under study. MICs were not significantly affected by increasing resistance to penicillin (*S. pneumoniae*), oxacillin (staphylococci), or vancomycin (enterococci) or the presence of ESBLs (*E. coli* and *K. pneumoniae*). Tentative disk diffusion breakpoints based upon provisional MIC breakpoints can be proposed for *S. pneumoniae* and *Haemophilus* spp., and FDA-approved breakpoints are confirmed for the other species. The following MIC and/or disk diffusion quality control ranges were established: *S. aureus* ATCC 29213, 0.03 to 0.25 µg/ml; *S. aureus* ATCC 25923, 20 to 25 mm; *E. coli* ATCC 25922, 0.03 to 0.25 µg/ml and 20 to 27 mm, *E. faecalis* ATCC 29212, 0.03 to 0.12 µg/ml; *P. aeruginosa* ATCC 27853, 9 to 13 mm; *S. pneumoniae* ATCC 49619, 0.015 to 0.12 µg/ml and 23 to 29 mm; *H. influenzae* ATCC 49247, 0.06 to 0.5 µg/ml and 23 to 31 mm; and *N. gonorrhoeae* ATCC 49226, 30 to 40 mm. These quality control ranges for both MIC and disk diffusion methodologies have been accepted and published by the CLSI and are in the package insert for tigecycline.

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