Multicenter Studies to Establish Tigecycline Disk Diffusion Susceptibility Breakpoints when Testing *Acinetobacter* spp.

C-172

RN JONES, MJ FERRARO, B RELLER, P SHRECKENBERGER, J SWENSON, HS SADER
JMI Laboratories, North Liberty, IA, USA; Massachusetts General Hospital, Boston, MA, USA; Duke University Medical Center, Durham, NC, USA; Loyola University Medical Center, Chicago, IL, USA; Centers for Disease Control and Prevention, Atlanta, GA, USA

ASM 2006
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

ABSTRACT

Background:
Acinetobacter spp. (ACB) isolates having multidrug resistant (MDR) patterns have become common in many medical centers, thus limiting therapeutic options. Tigecycline (TIG), a novel glycylcycline, has demonstrated *in vitro* activity against ACB, but interpretive criteria for broth microdilution (BMD) and disk diffusion (DD) methods have not been established for clinical laboratory (CL) use. Currently some CLs apply US-FDA package insert (PI) susceptibility (S) criteria for the Enterobacteriaceae (ENT).

Methods:
A 5-center study tested 103 contemporary ACB, including MDR strains by CLSI BMD (fresh MH broth) and DD (15-µg) methods against TIG. Also 133 ENT strains (US-FDA indicated species) were tested as controls of the published PI breakpoints (S/R at ≤2/≥8 µg/ml or ≥19/≤14 mm). QC was assured by concurrent use of *E. coli* ATCC 25922; all results were within CLSI limits (2005). Proposed breakpoints used US-FDA ENT MIC (≤2 µg/ml) and DD zones were selected to minimize intermethod discords by the error-rate bounding approach (NCCLS M23-A2), and to validate anecdotal reports of false-intermediate DD results.

Results:
ACB had TIG MICs ranging from 0.03 to 16 µg/ml and regression statistics (y=19.5-0.56x; r =0.87) demonstrated excellent correlations between BMD and DD results. Applying US-FDA TIG PI criteria (≤2 µg/ml) to ACB led to unacceptable error (23.3%); adjustment of TIG DD breakpoints (S/R) to ≥16/≤12 mm minimized errors to only 9.7% (all minor). Control ENT scattergrams for TIG using US-FDA DD breakpoints produced 18.8% error (18.0 minor; 0.8% major); this rate was corrected to only 3.0% minor errors by the proposed DD breakpoints for ACB testing.

Conclusions:
To address urgent CL needs for TIG breakpoint criteria for ACB, the US-FDA MIC used for ENT was suggested with candidate zones of ≥16 mm (S), resulting in intermethod errors that were reduced to acceptable levels. High intermethod errors appear to be occurring when testing TIG versus ENT (US-FDA DD breakpoint at ≥19 mm). TIG test results directing therapy should employ MIC methods (CLSI or Etest [AB BIODISK] preferred) or these proposed DD criteria for ACB.

INTRODUCTION

Acinetobacter spp. isolates have emerged in recent years among the most problematic pathogens to eradicate using available antimicrobial agents. The occurrences of *Acinetobacter* spp. infections have escalated in the National Nosocomial Infection Survey (ICARE) to levels of 6.9, 2.4, 2.1 and 1.6% as causes of healthcare associated pneumonia (HAP), bloodstream infections, surgical site infections and urinary tract infections, respectively. Similarly, the SENTRY Antimicrobial Surveillance Program lists *Acinetobacter* spp. as causing 2.3-3.0% of HAP and being the 8th most common organism (4.0%) isolated from ICU patients. Complicating this increased prevalence, multidrug resistance (MDR) among *Acinetobacter* spp. isolates has markedly risen because of acquired or selected mechanisms of resistance that include antimicrobial inactivation enzymes, efflux pumps, target modifications and altered porins.

Treatment of *Acinetobacter* spp. infection has largely been limited to a few broad-spectrum agents including carbapenems, amikacin, some tetracyclines (doxycycline and minocycline), and the sulbactam component of ampicillin/sulbactam. As resistance to the carbapenems and other alternatives has emerged, the popularity of polymyxin class agents (colistin and polymyxin B) has increased with documentation of clinical success, but not without side effects, usually renal toxicity (27-58%).

The therapeutic search has recently focused on a new class of antimicrobial agents, glycylcyclines, represented by tigecycline (a 9-t-butylglycylamide derivative of minocycline). Tigecycline has a novel, usually bactericidal, mode of action that binds to the 30S ribosomal subunit thus blocking amino-acyl tRNA entry to the acceptor site (e.g. no protein synthesis), an action that overcomes two types of tetracycline resistance (efflux and ribosomal protection). Tigecycline displays significant inhibitory activity against *Acinetobacter* spp., and has been utilized for therapy against MDR strains without interpretive criteria for in vitro susceptibility testing. This presentation summarizes results from a multi-center investigation of tigecycline tested by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]) methods to address needed susceptibility breakpoints for *Acinetobacter* spp., and to address the perception that applying the United States-Food and Drug Administration (US-FDA) tigecycline breakpoints used for Enterobacteriaceae to *Acinetobacter* spp. results in unacceptable error, that being false resistance by disk diffusion tests (personal communication, Wyeth Research).

MATERIALS AND METHODS

The study was designed to utilize five laboratories with *Acinetobacter* spp. isolates derived from the collection of clinical strains at each geographically diverse location. A total of 103 strains were identified locally (60 *A. baumannii*, two *A. lwoffii*, 41 other unspecified *Acinetobacter* species), and susceptibility tested by CLSI methods using freshly prepared cation-adjusted Mueller-Hinton medium for frozen-form broth microdilution tests (prepared by TREK Diagnostics, Cleveland, Ohio), and 15-µg tigecycline disks (BBL lot no 5187044; Sparks, Maryland) for the disk diffusion procedure. Each institution provided the current Mueller-Hinton agar lot in-use at that facility. Quality control (QC) strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) were concurrently tested on ≥ five occasions by each participant for tigecycline and control agents (gentamicin, tetracycline and tobramycin); all results (100.0%) except gentamicin (96.2% by MICs only) were within QC ranges recommended by the CLSI. The inoculum colony counts for the broth microdilution method averaged 3.8 x 10^5 CFU/ml across all participant sites. This protocol design conforms to the CLSI M23-A2 document recommendations.

Additional Enterobacteriaceae isolates of indicated species (*E. coli*, *Klebsiella pneumoniae*, *K. oxytoca*, *Citrobacter freundii* and *Enterobacter cloacae* [164 strains]) were tested as described for *Acinetobacter* spp. and analyzed for intermethod error rates.

Initial analyses examined tigecycline MIC and zone diameter results for serious discords (susceptible to resistant or vice versa) and tests were repeated to assess reproducibility. The two occurrences of intermethod discord resolved on repeat testing and the entire collection (103 strains) was analyzed using the US-FDA tigecycline susceptible breakpoint for Enterobacteriaceae (≤2 µg/ml and ≥19 mm) applied to the *Acinetobacter* spp. Resistance was defined as ≥8 µg/ml and ≤14 mm (see Table 1). The scattergram comparing tigecycline MIC and zone diameters around 15-µg disk results was constructed (Figure 1) and analyzed by the error rate bounding method to maximize intermethod agreement for the MIC breakpoint at ≤2 µg/ml. Generally, the goal of such calculations should minimize false-susceptible (very major) errors for the disk diffusion test to ≤1.5% as well as, intermethod minor and total error to ≤10.0%. Also, the tigecycline MIC distribution and percentage of *Acinetobacter* spp. strains inhibited at ≤2 µg/ml for the collection was compared to that reported from four distinct surveillance reports.

ACKNOWLEDGEMENTS

The co-authors appreciate the excellent support provided at each center by numerous technologists and managers. Also, the following individuals contributed to the development of the protocol and manuscript draft: N. O'Mara-Morrissey, J. Ross, M. Dowzicky, T. Fritsche, and P. Bradford. This study and the associated work of the CLSI *Acinetobacter* Working Group was funded in part by an educational/research grant from Wyeth Research.

SELECTED REFERENCES

1. Bradford PA (2004). Tigecycline: A first in class glycycycline. *Clin Microbiol Newsletter* 26: 163-168.
2. Clinical and Laboratory Standards Institute. (2006). *Performance standards for antimicrobial susceptibility testing, 16th informational supplement M100-S16*. Wayne, PA: CLSI.
3. Clinical and Laboratory Standards Institute. (2006). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. Approved Standard M7-A7*. Wayne, PA: CLSI, 2006.
4. Clinical and Laboratory Standards Institute. (2006). *Performance standards for antimicrobial disk susceptibility tests 9th ed. Approved standard M2-A9*. Wayne, PA: CLSI.
5. Fournier PE, Richet H (2006). The epidemiology and control of *Acinetobacter baumannii* in health care facilities. *Clin Infect Dis* 42: 692-699.
6. Fritsche TR, Sader HS, Stilwell MG, Dowzicky MJ, Jones RN (2005). Antimicrobial activity of tigecycline tested against organisms causing community-acquired respiratory tract infection and nosocomial pneumonia. *Diagn Microbiol Infect Dis* 52: 187-193.
7. Fritsche TR, Sader HS, Stilwell MG, Dowzicky MJ, Jones RN (2005). Potency and spectrum of tigecycline tested against an international collection of bacterial pathogens associated with skin and soft tissue infections (2000-2004). *Diagn Microbiol Infect Dis* 52: 195-201.
8. Jones RN (1999). Disk diffusion susceptibility test development for the new glycylcycline, GAR-936. *Diagn Microbiol Infect Dis* 35: 249-252.
9. Levin AS (2003). Treatment of *Acinetobacter* spp. infections. *Expert Opin Pharmacother* 4: 1289-1296.
10. Nathwani D (2005). Tigecycline: Clinical evidence and formulary positioning. *Int J Antimicrob Agents* 25: 185-192.
11. National Committee for Clinical Laboratory Standards. (2001). *Approved guideline M23-A2: Development of in vitro susceptibility testing criteria and quality controls parameters, 2nd ed*. Wayne, PA: NCCLS.
12. Petersen PJ, Bradford PA (2005). Effect of medium age and supplementation with the biocatalytic oxygen-reducing reagent oxyrase on in vitro activities of tigecycline against recent clinical isolates. *Antimicrob Agents Chemother* 49: 3910-3918.
13. Petersen PJ, Bradford PA, Weiss WJ, Murphy TM, Sum PE, Projan SJ (2002). In vitro and in vivo activities of tigecycline (GAR-936), daptomycin, and comparative antimicrobial agents against glycopeptide-intermediate *Staphylococcus aureus* and other resistant gram-positive pathogens. *Antimicrob Agents Chemother* 46: 2595-2601.
14. Sader HS, Jones RN, Dowzicky MJ, Fritsche TR (2005). Antimicrobial activity of tigecycline tested against nosocomial bacterial pathogens from patients hospitalized in the intensive care unit. *Diagn Microbiol Infect Dis* 52: 203-208.
15. Sader HS, Jones RN, Stilwell MG, Dowzicky MJ, Fritsche TR (2005). Tigecycline activity tested against 26,474 bloodstream infection isolates: A collection from 6 continents. *Diagn Microbiol Infect Dis* 52: 181-186.
16. Talbot GH, Bradley J, Edwards JE, Jr., Gilbert D, Scheld M, Bartlett JG (2006). Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clin Infect Dis* 42: 657-668.
17. Tygacil Product Insert. 2005. Philadelphia (PA): Wyeth Pharmaceuticals Inc. (June, 2005).

RESULTS

- Figure 1 shows the scattergram of tigecycline MIC results to zone diameters around 15-µg disks. The *Acinetobacter* spp. (103) demonstrated a very linear correlation (r = 0.87) between the results, with only three strains having a tigecycline MIC at ≥8 µg/ml. Using the US-FDA susceptible breakpoint of ≤2 µg of tigecycline/ml (Enterobacteriaceae) for this collection with the correlate zones, unacceptably high minor error rate (23.3%) was observed (Table 1).

- By modifying the susceptible and resistant breakpoint zones to ≥16 and ≤12 mm (Table 1), respectively; the minor and total error rates were minimized to 9.7%. These findings, using the US-FDA tigecycline package insert breakpoints for the disk diffusion method, are consistent with the anecdotal reports of high numbers of "tigecycline-intermediate" *Acinetobacter* spp. isolates when using the "Kirby-Bauer" method.

- Tigecycline disk diffusion breakpoints were slightly more tigecycline-resistant (2.9%) than the published data for more than 800 strains (0.0-1.6% resistant; Table 2). The tigecycline MIC_{90} results ranged from 2-4 µg/ml and percentages of isolates with a tigecycline MIC at ≤2 µg/ml was 86.7-96.7%. Compared to these surveillance program results, the five-center study collections were judged as representative of current USA clinical material/strains.

- Tests with Enterobacteriaceae strains revealed an intermethod error rate of 15.8% (15.2% minor; 0.6% major) using disk diffusion breakpoints found in the tigecycline US-FDA package insert (data not shown). Using modified breakpoints as proposed for *Acinetobacters*, produced 97.6% absolute intermethod categorical agreement (only 2.4% minor errors). Re-evaluation of the Enterobacteriaceae tigecycline susceptible criteria for the disk diffusion test seems urgently needed.

Table 1. Proposed tigecycline interpretive criteria for testing *Acinetobacter* spp. by CLSI methods and the calculated inter-method error rates for the disk diffusion method derived from the scattergram presented in Figure 1.

MIC (µg/ml) Interpretative criteria (correlate zones)			Error rates (%)		
Susceptible	Intermediate	Resistant	Very major	Major	Minor
≤2 (≥19)[b]	4 (15-18)	≥8 (≤14)	0.0	0.0	23.3%
≤2 (≥16)[b]	4 (13-15)	≥8 (≤12)	0.0	0.0	9.7%[c]
≤2 (≥17)	4 (14-16)	≥8 (≤13)	0.0	0.0	11.7%

a. Indicates MIC and disk diffusion criteria found for Enterobacteriaceae in the tigecycline product package insert.
b. Criteria that minimize intermethod error rates by modifying the disk diffusion test criteria only.
c. Acceptable level of intermethod discord.

Table 2. Summary of published tigecycline activity results tested against 773 *Acinetobacter* spp. isolates among 51,619 strains reported from an international surveillance program and 49 additional bacteremias.

Organism source	Tigecycline MIC (µg/ml)			% at MIC:		% by category[b]	
(no. tested/%)[a]	Reference	50%	90%	≤1 µg/ml	≤2 µg/ml	Susceptible	Resistant
Bacteremia (326/1.2%)	21	0.5	2	74	95	94.5	0.9
Bacteremia (49/NA)	18	2	2	-	92	91.8	0.0
ICU (223/2.4%)	19	1	2	65	93	93.3	0.9
Respiratory tract (143/4.5%)	8	1	4	51	87	86.7	0.7
SSTI (61/1.2%)	7	0.5	2	87	97	96.7	1.6

a. Totals for 773 strains with the percentage of *Acinetobacter* spp. isolates among the total species reported for each specimen source
b. Categories were defined using the US-FDA tigecycline package insert criteria for Enterobacteriaceae (≤2 µg/ml as susceptible). Resistance was defined as a tigecycline MIC at ≥8 µg/ml.

Figure 1. Scattergram comparing tigecycline MIC values (µg/ml) and zones of inhibition around 15-µg tigecycline disks when tested against 103 *Acinetobacter* isolates. This was a multi-center (five sites) investigation with a diverse collection of recent clinical strains. Solid vertical and horizontal lines show the interpretive criteria for Enterobacteriaceae published in the US-FDA approved product package insert when using CLSI methods. Broken vertical and horizontal lines illustrate the proposed breakpoints for *Acinetobacter* spp. testing among two options (see Table 1).

CONCLUSIONS

- Like its parent compound (minocycline), tigecycline exhibits potent activity against *Acinetobacter* spp. comparable to that shown against the indicated species of Enterobacteriaceae (Table 2). Therefore, suggested susceptibility breakpoints for *Acinetobacter* spp. and Enterobacteriaceae should be consistent, as well as conforming, where possible to the product package insert. A modest change in the tigecycline 15-µg disk diffusion breakpoints (Table 1) by only 3 mm improves the tigecycline intermethod agreement and predictive accuracy to acceptable levels.

- Those laboratories asked to provide tigecycline in vitro susceptibility testing for *Acinetobacter* spp. isolates should attempt to comply by using a validated quantitative MIC method (broth microdilution or Etest [AB BIODISK, Solna, Sweden]), but when the disk diffusion test must be employed, these proposed breakpoint modifications should be considered to maximize interpretative accuracy.