C-054

Re-Evaluation of Disk Diffusion Breakpoints for Tigecycline When Testing Enterobacteriaceae

HS SADER, MJ FERRARO, LB RELLER, P SCHRECKENBERGER, J SWENSON, RN JONES
JMI Laboratories, Iowa City, IA; Massachusetts General Hospital, Boston, MA; Duke University Hospital, Durham, NC;
Loyola University Chicago, Chicago, IL; Center for Disease Control and Prevention, Atlanta, GA

ASM 2007
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

AMENDED ABSTRACT*

Background:

We re-evaluated US-FDA package insert (PI) disk diffusion (DD) method susceptibility criteria when testing tigecycline against Enterobacteriaceae (ENT; current breakpoints are ≥19 and ≤14 mm for susceptible (S) and resistant [R], respectively) to validate anecdotal reports of false-intermediate DD results, especially with multidrug-R (MDR) *Klebsiella* spp.

Methods:

350 recent clinical isolates were studied including *E. coli* (100), *K. pneumoniae* (KPN; 100), *K. oxytoca* (KOX; 50), *E. cloacae* (50), *C. freundii* (50). Isolates were tested by CLSI DD and broth microdilution (BMD) methods. Proposed breakpoints used US-FDA ENT MIC criteria (≤2 and ≥8 µg/ml for S and R, respectively) and correlate disk diffusion zone diameters were selected to minimize discords by error-rate bounding analyses (M23-A2). When hazy growth was present, zone diameters were read at the significant decrease in growth. Regression and scattergram plot analyses determined intermethod accuracy for current and proposed breakpoints.

Results:

Regression analysis showed a (r) value of 0.66. When tested by BMD, no tigecycline-resistant strain was observed and 5 strains (4 KPN and 1 ECL) showed tigecycline MIC of 4 µg/ml (intermediate). DD error rates were (minor[Mi]/major[Ma]/very major[VM]): 3.1/0.0/0.0% when using current US-FDA PI criteria; 81.8% (11 of 12) of Mi errors involved isolates categorized as S by broth microdilution and intermediate by DD. 8 of 11 (72.7%) Mi errors were noted for KPN isolates (8.0%). When breakpoints were adjusted to (S/R) ≥16/≤12 mm, no VM (false-S) or Ma (false-R) errors were observed and the Mi error rate was minimized to only 0.6% overall for the five indicated species.

	Error rates (%)			
	Current breakpoints (≥19 / ≤14 mm)		Proposed breakpoints (≥16 / ≤12 mm)	
Organism	Ma	Mi	Ma	Mi
C. freundii (50)	0.0	4.0	0.0	0.0
E. cloacae (50)	0.0	2.0	0.0	2.0
E. coli (100)	0.0	0.0	0.0	0.0
K. oxytoca (50)	0.0	0.0	0.0	0.0
K. pneumoniae (100)	0.0	8.0	0.0	1.0
Total (350)	0.0	3.1	0.0	0.6

Conclusions:

US-FDA should consider the proposed changes that significantly improve the accuracy of DD testing (especially for MDR *Klebsiella* spp.) with this new glycylcycline. Reading can be difficult due to the frequent presence of hazy growth at the margin of inhibition.

*The abstract was amended to reflect the correct number of errors (11) when using US-FDA breakpoints and the correct rate of minor errors (0.6%) when using adjusted breakpoints.

INTRODUCTION

Tigecycline has a novel, often bactericidal, mode of action characterized by binding to the 30S ribosomal subunit, thus blocking amino-acyl tRNA entry to the acceptor site (i.e. inhibiting protein synthesis), an action that overcomes two types of tetracycline resistance (efflux and ribosomal protection). Tigecycline displays good in vitro activity against most Enterobacteriaceae species and was approved by the United States Food and Drug Administration (US-FDA) and the European Medicines Agency (EMEA) for the treatment of complicated skin and soft tissue infections (cSSTI) and intra-abdominal infections (IAI). Furthermore, tigecycline has been shown to be active, both in vitro and in clinical infections, against most strains of the following Enterobacteriaceae species: *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca* and *Klebsiella pneumoniae*.

This report summarizes results from a multi-center investigation of tigecycline tested using the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]) methods to re-evaluate US-FDA package insert disk diffusion breakpoints when testing tigecycline against "indicated" Enterobacteriaceae species to validate anecdotal reports of false-intermediate disk diffusion results, especially with multidrug-resistant *Klebsiella* spp.

MATERIALS AND METHODS

The study was designed utilizing several laboratories with Enterobacteriaceae strains selected from contemporary clinical isolates at five geographically diverse locations. A total of 350 non-duplicated, recent clinical strains were identified locally. The collection includes: *E. coli* (100 strains), *K. pneumoniae* (100), *K. oxytoca* (50), *E. cloacae* (50), and *C. freundii* (50). Isolates were tested for susceptibility by CLS methods using freshly (<12 hours) prepared cation-adjusted Mueller-Hinton medium for frozen-form M7-A7 (2006) broth microdilution tests (TREK Diagnostics, Cleveland, Ohio). This protocol design conforms to the NCCLS M23-A2 document recommendations. Tigecycline disks were used (15-µg BBL lot no 5187044; Sparks, Maryland) for the disk diffusion procedure (M2-A9, 2006). Each institution utilized the current Mueller-Hinton agar lot in use at that facility.

MIC breakpoints used US-FDA Enterobacteriaceae criteria (≤2 and ≥8 µg/ml for susceptibility and resistance, respectively) and correlate disk diffusion zone diameters were those found in the tigecycline product package insert and others selected to minimize discords by error-rate bounding analyses (M23-A2). When hazy growth was present, zone diameters were read as the significant decrease in growth. Regression and scattergram plot analyses determined intermethod agreement for current and proposed breakpoints.

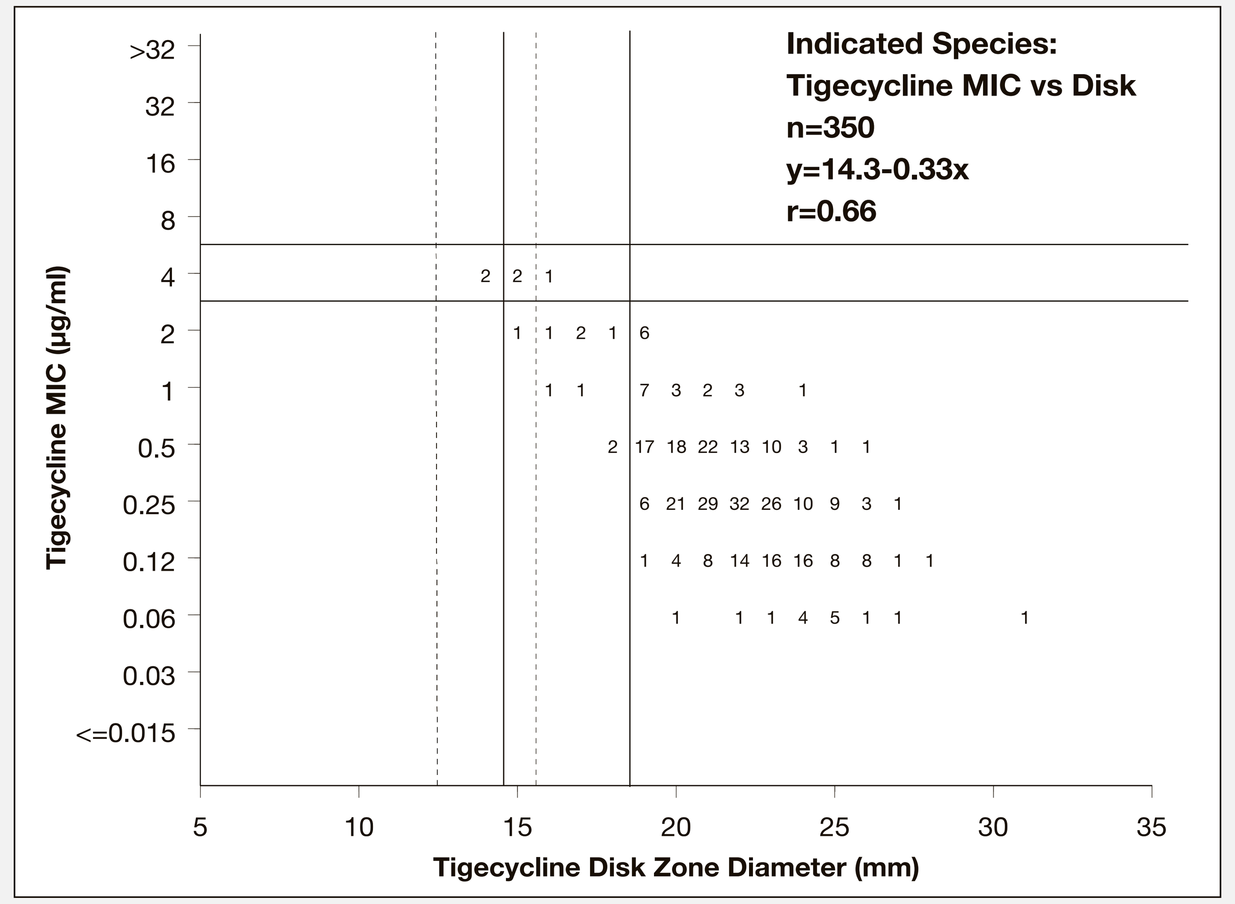
ACKNOWLEDGEMENTS

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 abstract: N. O'Mara-Morrissey, J. Ross, M. Dowzicky, T. Fritsche, and P. Bradford. This study was funded in part by an educational/research grant from Wyeth Research.

RESULTS

- Figure 1 shows the scattergram of tigecycline MIC results compared to zone diameters around 15-µg disks for all indicated species tested. The 350 Enterobacteriaceae strains demonstrated a favorable linear correlation (r = 0.66).
- When tested by broth microdilution methods, no tigecycline-resistant (MIC at ≥8 µg/ml) strains were observed and five strains (four *K. pneumoniae* and one *E. cloacae*) showed a tigecycline MIC of 4 µg/ml (intermediate; Figures 2 and 3).
- Disk diffusion results exhibited 3.1% minor errors and no very major (false-susceptible) or major (false-resistant) errors when using current US-FDA package insert criteria (solid vertical lines). Nine of 11 (83.3%) minor errors involved isolates categorized as susceptible by broth microdilution method and intermediate by disk diffusion method, and eight of 11 (72.7%) minor errors were noted for *K. pneumoniae* isolates (Figures 1 and 2).
- When disk diffusion breakpoints were adjusted to ≥16 mm for susceptible and ≤12 mm for resistant (broken vertical lines), the minor error rate was minimized to only 0.6% overall with no very major or major errors. The discrepant results (minor errors) were again observed with *K. pneumoniae* (one strain) and *E. cloacae* (one strain; Figures 1 to 4).

Figure 1. Scattergram showing the correlation between disk diffusion zone diameters and MIC values for all indicated species grouped together.



- Hazy growth at the margin of the inhibitory zone was frequently observed.

Figure 2. Scattergram showing the correlation between disk diffusion zone diameters and MIC values for *Klebsiella pneumoniae*.

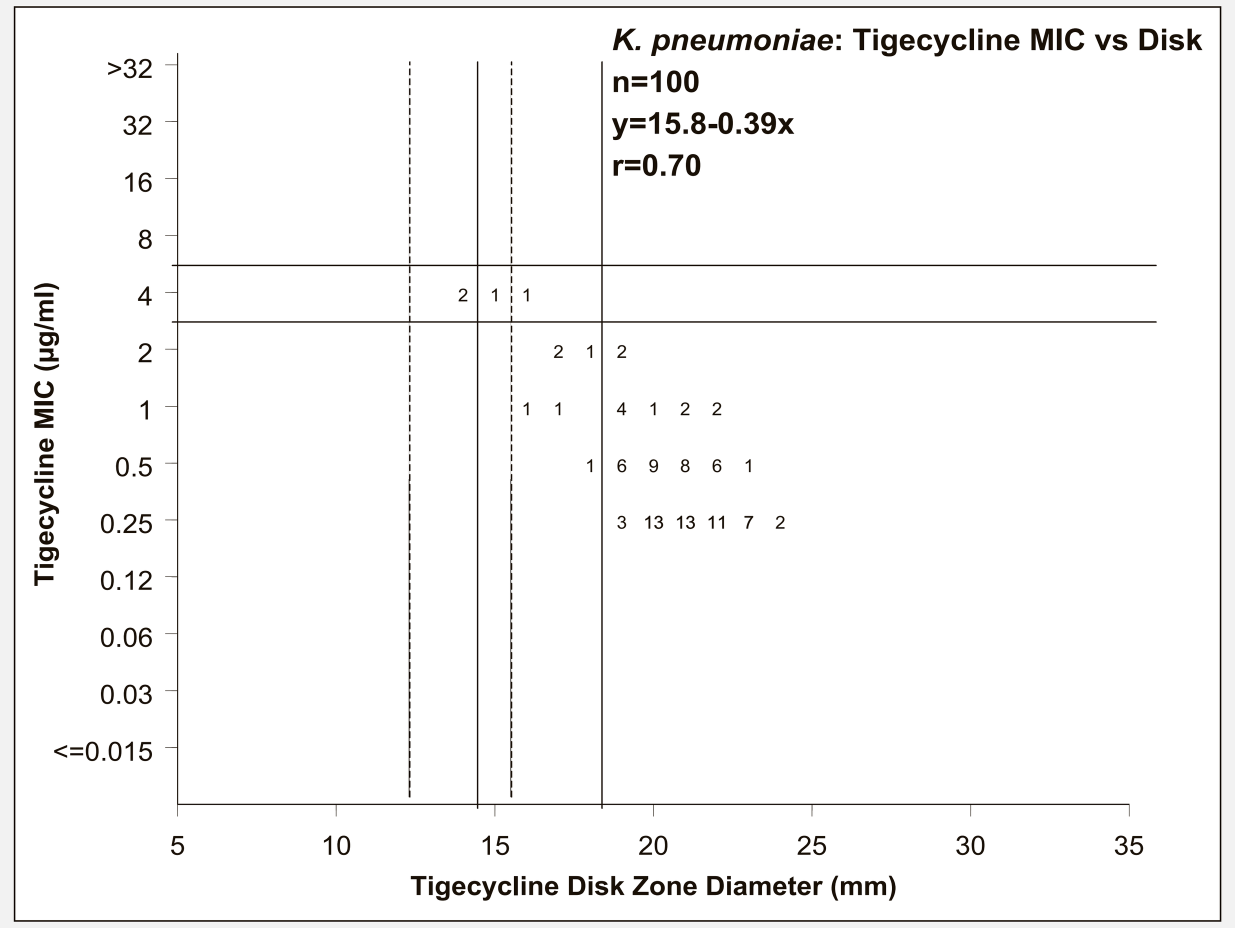
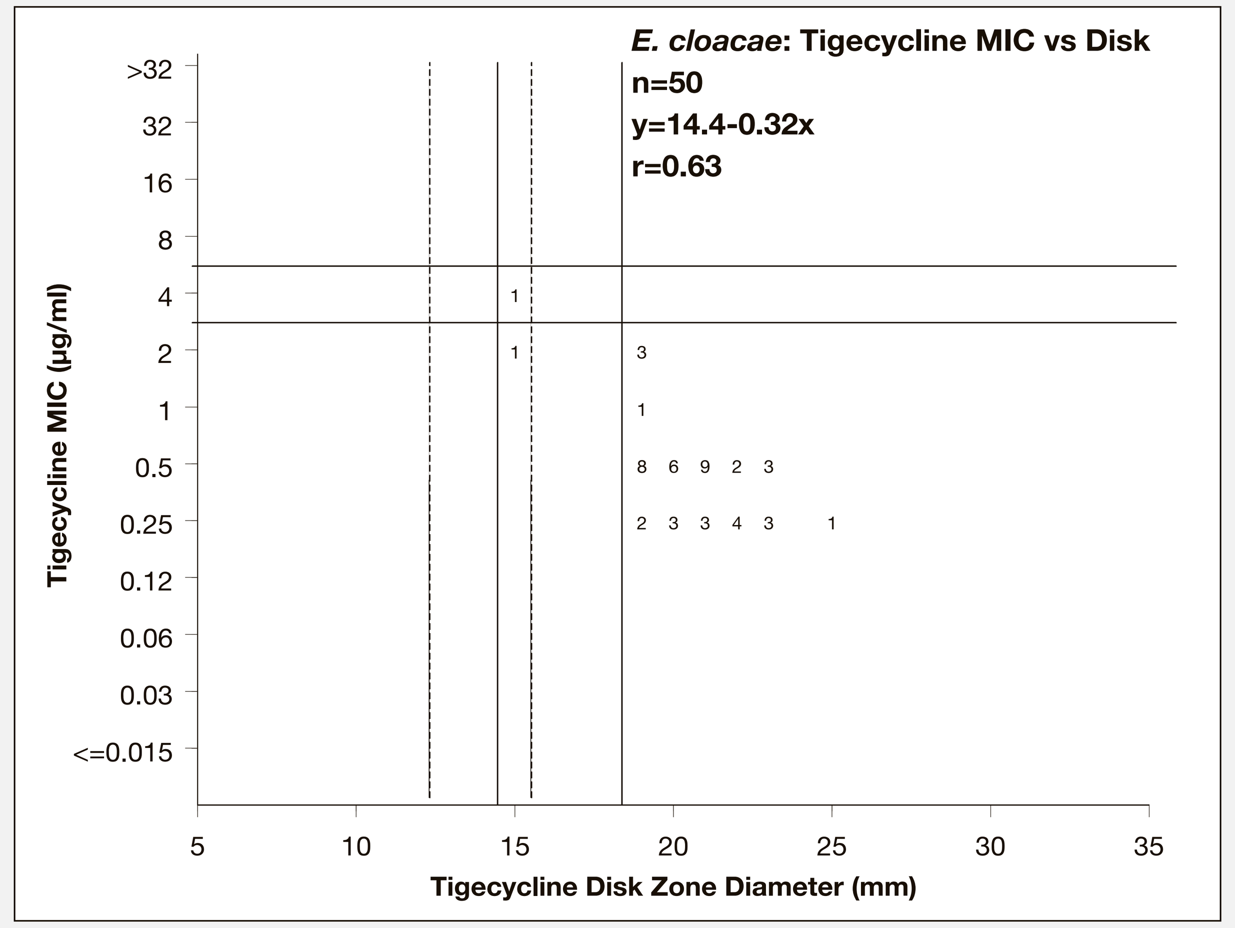
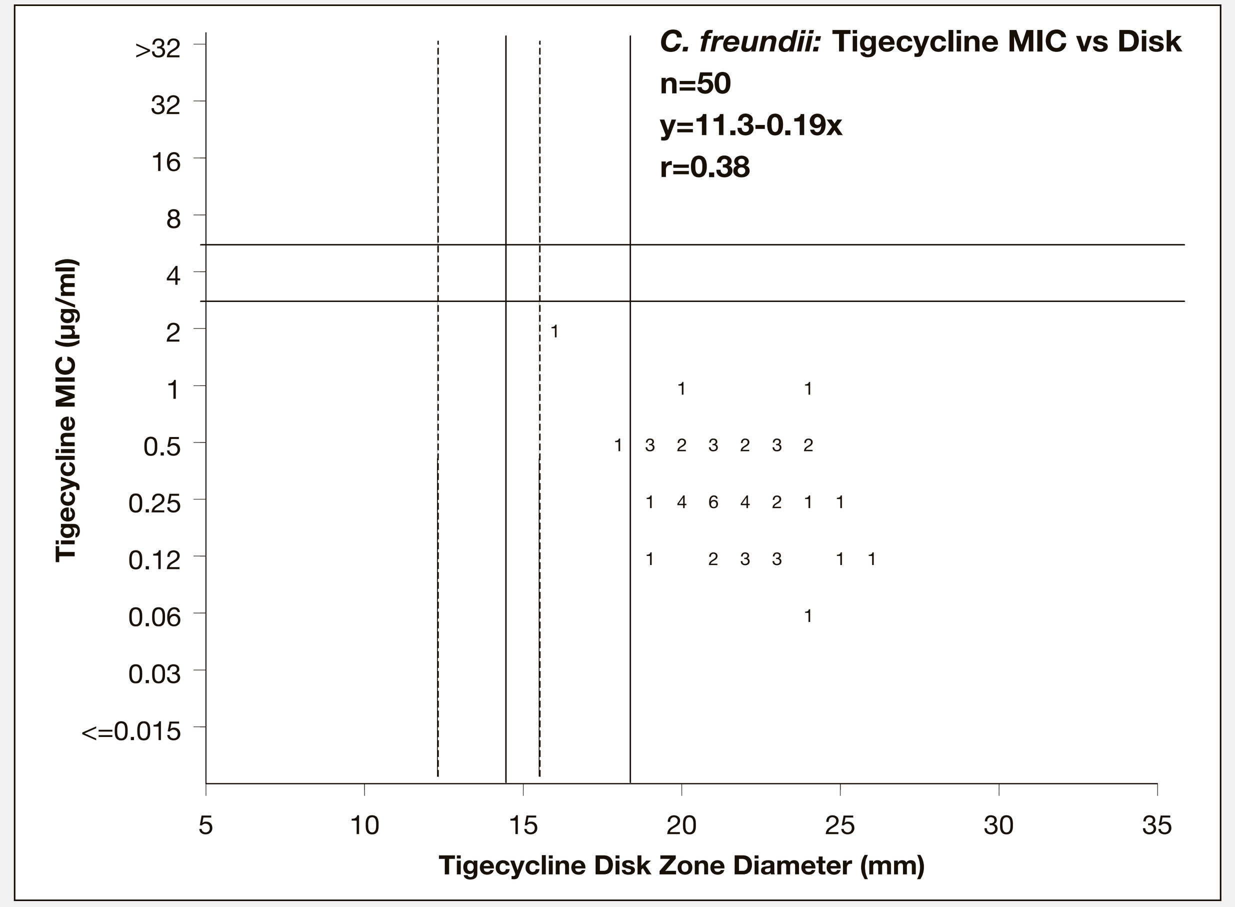


Figure 3. Scattergram showing the correlation between disk diffusion zone diameters and MIC values for *Enterobacter cloacae*.



- Our results are substantiated by a recent study involving 4,111 Enterobacteriaceae strains from the Asia-Pacific region. The strains were tested by disk diffusion and broth microdilution methods. When US-FDA breakpoints were applied categorical agreement for indicated species (1,841 strains) was 89.6% and disk diffusion results showed a clear tendency toward more resistance to tigecycline. Discordant results were minimized to 0.8% (only minor errors) when the breakpoints proposed here were applied.

Figure 4. Scattergram showing the correlation between disk diffusion zone diameters and MIC values for *Citrobacter freundii*.



CONCLUSIONS

- US-FDA disk diffusion testing criteria for tigecycline should be reconsidered using the proposed changes that significantly improve the test accuracy (especially for multidrug-resistant *Klebsiella* spp.).
- Reading the disk diffusion inhibition zone can be difficult due to the frequent presence of hazy growth at the margin of inhibition.
- These results confirm a systematic trend toward tigecycline false-intermediate interpretations using the disk diffusion method and current US-FDA breakpoint criteria.