

Quality Control MIC Ranges Used for Telavancin with Application of a Revised CLSI Reference Broth Microdilution Method

James E. Ross, Rodrigo E. Mendes, Ronald N. Jones

JMI Laboratories, North Liberty, Iowa, USA

The telavancin broth microdilution susceptibility testing method was revised, which provides MIC results lower than those obtained by the previous method. This study was performed to reestablish the quality control ranges for telavancin when tested against the strains (updated MIC range) *Staphylococcus aureus* ATCC 29213 (0.03 to 0.12 µg/ml), *Enterococcus faecalis* ATCC 29212 (0.03 to 0.12 µg/ml), and *Streptococcus pneumoniae* ATCC 49619 (0.004 to 0.015 µg/ml).

Telavancin is a lipoglycopeptide antibiotic approved in the United States and Canada for the treatment of patients with complicated skin and skin structure infections due to susceptible Gram-positive pathogens and in the United States and Europe for the treatment of hospital-acquired bacterial pneumonia, including ventilator-associated bacterial pneumonia (HABP/VABP) due to susceptible isolates of *Staphylococcus aureus* (methicillin-resistant *Staphylococcus aureus* [MRSA] strains only in Europe), when alternative medicines are unsuitable (1). Telavancin is active against nearly all clinically important Gram-positive bacteria: staphylococci (including methicillin-resistant and vancomycin-intermediate strains), streptococci (including multidrug-resistant pneumococci), enterococci (vancomycin-susceptible isolates only), Gram-positive anaerobes such as members of the genus *Clostridium* (including *Clostridium difficile*), and other less commonly encountered Gram-positive pathogens (2, 3).

Previous CLSI supplemental documents updated annually (M100-S15 through M100-S23 [4]) recommended the use of dimethyl sulfoxide (DMSO) and water as solvent and diluent, respectively, for preparations of stock solutions and drug dilutions for manufacturing 96-well frozen-form panels for telavancin susceptibility testing. This method was used to establish the previous telavancin MIC quality control (QC) ranges (4). However, the broth microdilution (BMD) method for telavancin has been revised, and now consists of using DMSO as the solvent and the diluent, following the CLSI guidelines for stock solution and dilution preparations of water-insoluble agents (see Table 8B in reference 5). Moreover, similar to dalbavancin and oritavancin, this modified method for telavancin includes the addition of polysorbate-80 (P-80) (0.002%) to the Mueller-Hinton test medium to minimize drug-binding loss to plastic surfaces (6–8).

These changes were shown to improve the solubility of the drug during panel preparation (DMSO) and drug availability in the 96-well plastic plates (P-80), resulting in a more accurate and reproducible *in vitro* assessment of telavancin MIC determinations (Theravance, Inc., data on file [study no. THV-08-001, study no. THV-07-001, and report 09-6424-MCB-01]). In addition, studies conducted during the development of the revised method demonstrated that the MICs for telavancin when tested against staphylococci and enterococci were 4- to 8-fold lower than those obtained by the previous CLSI-recommended method (Theravance, Inc., data on file [report 08-THE-03]). The differences in the telavancin MIC values obtained by both methods were less pronounced when tested against streptococci. Therefore, this

TABLE 1 Quality control MIC ranges for telavancin when tested against ATCC strains using the previous and revised broth microdilution methods

QC organism	MIC (µg/ml)	No. (%) of replicates inhibited at telavancin MIC in ^a :	
		Revised method ^b	Previous method ^c
<i>S. aureus</i> ATCC 29213	1		17 (7.1)
	0.5		71 (29.6)
	0.25		151 (62.9)
	0.12	3 (0.9)	1 (0.4)
	0.06	228 (71.3)	
	0.03	89 (27.8)	
<i>E. faecalis</i> ATCC 29212	1		1 (0.4)
	0.5		34 (14.2)
	0.25		203 (84.6)
	0.12	51 (15.9)	2 (0.8)
	0.06	265 (82.8)	
	0.03	4 (1.3)	
<i>S. pneumoniae</i> ATCC 49619	0.03		1 (0.4)
	0.015	45 (14.1)	22 (9.2)
	0.008	268 (83.8)	116 (48.3)
	0.004	7 (2.2)	101 (42.1)
	0.002		

^a Results in bold type represent the MIC QC ranges for the revised susceptibility testing method approved by the CLSI (document M100-S24 [5]) and the previous method (document M100-S23 [4] and the earlier versions [documents M100-S15 through M100-S22]).

^b MIC QC ranges for the revised method proposed and approved by the CLSI (document M100-S24[5]). All values were within the proposed ranges.

^c MIC QC ranges proposed for the previous method were 0.12 to 1 µg/ml (*S. aureus* ATCC 29213) (all MICs within range), 0.12 to 0.5 µg/ml (*E. faecalis* ATCC 29212) (99.6% of MICs within range), and 0.002 to 0.015 µg/ml (*S. pneumoniae* ATCC 49619) (99.6% of MICs within range) (S. Brown, minutes of the NCCLS Antimicrobial Susceptibility Subcommittee meeting, 11 January 2004, Tempe, AZ).

Received 25 April 2014 Returned for modification 29 May 2014

Accepted 4 June 2014

Published ahead of print 11 June 2014

Editor: R. Patel

Address correspondence to James E. Ross, jim-ross@jmlabs.com.

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doi:10.1128/JCM.01210-14

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TABLE 2 Medium lot and inter- and intra-laboratory comparisons of telavancin MIC results obtained when tested against the listed ATCC QC strains.

ATCC strain and MIC	No. of occurrences for medium lot:				No. of occurrences for laboratory:								Total no.
	A	B	C	D	A	B	C	D	E	F	G	H	
<i>S. aureus</i> 29213													
0.03 µg/ml	11	30	11	37	12	27		7	8	8	16	11	89
0.06 µg/ml	68	50	67	43	28	12	40	33	31	31	24	29	228
0.12 µg/ml	1		2			1			1	1			3
Total	80	80	80	80	40	40	40	40	40	40	40	40	320
Mode	0.06	0.06	0.06	0.06	0.06	0.03	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Geomean	0.06	0.05	0.06	0.04	0.05	0.04	0.06	0.05	0.05	0.05	0.05	0.05	0.05
Log ₂ dilution range	3	2	3	2	2	3	1	2	3	3	2	2	3
<i>E. faecalis</i> 29212													
0.03 µg/ml				4		2		1				1	4
0.06 µg/ml	73	71	49	72	38	37	37	31	35	23	28	36	265
0.12 µg/ml	7	9	31	4	2	1	3	8	5	17	12	3	51
Total	80	80	80	80	40	40	40	40	40	40	40	40	320
Mode	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Geomean	0.06	0.06	0.08	0.06	0.06	0.06	0.06	0.07	0.07	0.08	0.07	0.06	0.07
Log ₂ dilution range	2	2	2	3	2	3	2	3	2	2	2	3	3
<i>S. pneumoniae</i> 49619													
0.004 µg/ml	2	2	3			1	6						7
0.008 µg/ml	67	72	70	59	23	33	29	34	33	40	40	36	268
0.015 µg/ml	11	6	7	21	17	6	5	6	7			4	45
Total	80	80	80	80	40	40	40	40	40	40	40	40	320
Mode	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Geomean	0.009	0.008	0.008	0.010	0.011	0.009	0.008	0.009	0.009	0.008	0.008	0.009	0.009
Log ₂ dilution range	3	3	3	2	2	3	3	2	2	1	1	2	3

study was conducted to reestablish the MIC QC ranges for telavancin when utilizing the revised BMD method.

The study presented here was performed according to the guidelines found in the CLSI M23-A3 document (9), which specifies the use of at least seven laboratories and three different manufacturers of media. This study utilized eight laboratories and four different media lots. Vancomycin was included as a control agent. BMD frozen-form panels were manufactured according to the modified method by Thermo Fisher Scientific (formerly TREK Diagnostics Systems/Sensititre, Cleveland, OH, USA). Results are presented as QC MIC ranges (µg/ml) for three strains (*S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Streptococcus pneumoniae* ATCC 49619). The telavancin MIC testing range for *S. aureus* and *E. faecalis* was 0.008 to 8 µg/ml, and that for *S. pneumoniae* was 0.001 to 1 µg/ml.

The eight laboratories were experienced clinical and/or research microbiology facilities and followed the CLSI procedures for BMD methods (10). The participating sites (and their principal investigator) were Massachusetts General Hospital, Boston, MA (M. J. Ferraro), Wheaton Franciscan Laboratory, Wauwatosa, WI (E. Munson), JMI Laboratories, North Liberty, IA (R. N. Jones), Thermo Fisher Scientific, Cleveland, OH (C. Knapp), University of Alberta, Edmonton, Alberta, Canada (R. Rennie), University of Washington, Seattle, WA (S. Swanzy), Duke University

Medical Center, Durham, NC (S. Mirrett), and Robert Wood Johnson Medical School, New Brunswick, NJ (M. Weinstein). Inoculum colony counts (CFU/ml) were performed on drug-free agar media and resulted in average counts of 2.8×10^5 CFU/ml (*S. aureus* ATCC 29213), 2.8×10^5 CFU/ml (*E. faecalis* ATCC 29212), and 2.5×10^5 CFU/ml (*S. pneumoniae* ATCC 49619). All results were considered acceptable.

The telavancin MIC ranges obtained against ATCC QC strains by the revised and previous BMD methods are summarized in Table 1. Telavancin displayed MIC results between 0.03 and 0.12 µg/ml, with an overall modal MIC value of 0.06 µg/ml (71.3% of MIC values) when tested against *S. aureus* ATCC 29213 (Tables 1 and 2). These telavancin modal MIC and MIC ranges were 4-fold lower and one doubling dilution narrower than those documented when using the previous method (modal MIC value of 0.25 µg/ml [62.9% of MIC values] and MIC range of 0.12 to 1 µg/ml) (4; S. Brown, minutes of the NCCLS Antimicrobial Susceptibility Subcommittee meeting, 11 January 2004, Tempe, AZ). Additional data analysis was performed using the Range Finder program (12), which confirmed the modal MIC and proposed a QC range for *S. aureus* of 0.03 to 0.12 µg/ml. Telavancin MIC results obtained against the *E. faecalis* ATCC 29212 strain were also within a 3-log₂ dilution range (0.03 to 0.12 µg/ml) with a

modal MIC value of 0.06 µg/ml; results confirmed by the Range Finder program (12).

S. pneumoniae ATCC 49619 had telavancin MIC results within 0.004 to 0.015 µg/ml, with an overall modal MIC value of 0.008 µg/ml. These telavancin MIC results obtained against *S. pneumoniae* ATCC 49619 with the revised method suggested a QC range and modal MIC value similar to those obtained with the previous testing methodology (0.004 to 0.03 µg/ml and 0.008 µg/ml, respectively) (Table 1). However, a greater reproducibility (83.8% versus 48.3% of MIC values at 0.008 µg/ml obtained by the revised and previous methods, respectively) and narrower range was obtained using the revised methodology. The Range Finder calculations confirmed the absence of outlier results, and the tool also suggested an MIC QC range of 0.004 to 0.015 µg/ml. All broth medium lots and laboratories shared the same modal MIC value, regardless of the ATCC strain tested (except for laboratory B versus *S. aureus* ATCC 29213, where the mode was 0.03 µg/ml [Table 2]). All vancomycin results were within published QC ranges providing a valid internal control (data not shown).

Overall, the updated MIC QC ranges described in this study are narrower (and more reproducible for *S. aureus* and *S. pneumoniae* ATCC strains) than those published for telavancin in the M100-S23 and earlier documents (4). Similar observations were reported for dalbavancin and oritavancin, when comparable method modifications were applied to the MIC testing for those agents (6–8). These updated telavancin QC ranges were approved by CLSI and published in the CLSI M100-S24 (2014) document (5). In addition, these data were reviewed and approved as part of a labeling supplement for the product Vibativ (telavancin) by the U.S. Food and Drug Administration. The revised testing method and the QC MIC ranges presented here, as well as updated interpretive breakpoints, have been included in the updated labeling (1). The reestablished QC ranges and associated breakpoints should now be applied for interpretations of telavancin MIC results obtained against QC and clinical isolates when using this revised CLSI BMD method (1, 5).

ACKNOWLEDGMENTS

This study was sponsored by an educational/research grant from Theravance, Inc. (South San Francisco, CA). J. E. Ross, R. E. Mendes, and R. N. Jones are employees of JMI Laboratories who receive grant funds to study telavancin and were paid consultants to Theravance in connection with the development of the manuscript.

We thank the eight contributing laboratories (personnel and direc-

tors) for their excellent support of this protocol. Coordination of scientific review of the draft manuscript by Theravance and partners was conducted by Suzanne Douthwaite, an employee of Envision Scientific Solutions, funded by Theravance.

REFERENCES

1. Theravance Inc. 2014. Vibativ package insert. Theravance, Inc., San Francisco, CA. <http://www.vibativ.com>.
2. Putnam SD, Sader HS, Moet GJ, Mendes RE, Jones RN. 2010. Worldwide summary of telavancin spectrum and potency against Gram-positive pathogens: 2007 to 2008 surveillance results. *Diagn. Microbiol. Infect. Dis.* 67:359–368. <http://dx.doi.org/10.1016/j.diagmicrobio.2010.03.009>.
3. Mendes RE, Sader HS, Farrell DJ, Jones RN. 2012. Worldwide appraisal and update (2010) of telavancin activity tested against a collection of Gram-positive clinical pathogens from five continents. *Antimicrob. Agents Chemother.* 56:3999–4004. <http://dx.doi.org/10.1128/AAC.00011-12>.
4. Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial susceptibility testing; 23rd informational supplement. CLSI M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.
5. Clinical and Laboratory Standards Institute. 2014. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
6. Arhin FF, Sarmiento I, Belley A, McKay GA, Draghi DC, Grover P, Sahn DF, Parr TR, Jr, Moeck G. 2008. Effect of polysorbate 80 on oritavancin binding to plastic surfaces: implications for susceptibility testing. *Antimicrob. Agents Chemother.* 52:1597–1603. <http://dx.doi.org/10.1128/AAC.01513-07>.
7. Arhin FF, Tomfohrde K, Draghi DC, Aranza M, Parr TR, Jr, Sahn DF, Moeck G. 2008. Newly defined *in vitro* quality control ranges for oritavancin broth microdilution testing and impact of variation in testing parameters. *Diagn. Microbiol. Infect. Dis.* 62:92–95. <http://dx.doi.org/10.1016/j.diagmicrobio.2008.05.009>.
8. Rennie RP, Koeth L, Jones RN, Fritsche TR, Knapp CC, Killian SB, Goldstein BP. 2007. Factors influencing broth microdilution antimicrobial susceptibility test results for dalbavancin, a new glycopeptide agent. *J. Clin. Microbiol.* 45:3151–3154. <http://dx.doi.org/10.1128/JCM.02411-06>.
9. Clinical and Laboratory Standards Institute. 2008. Development of *in vitro* susceptibility testing criteria and quality control parameters, 3rd ed. CLSI M23-A3. Clinical and Laboratory Standards Institute, Wayne, PA.
10. Clinical and Laboratory Standards Institute. 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 9th ed. CLSI M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
11. Reference deleted.
12. Turnidge J, Bordash G. 2007. Statistical methods for establishing quality control ranges for antibacterial agents in Clinical and Laboratory Standards Institute susceptibility testing. *Antimicrob. Agents Chemother.* 51:2483–2488. <http://dx.doi.org/10.1128/AAC.01457-06>.