Revised Reference Broth Microdilution Method for Testing Telavancin: Effect on MIC Results and Correlation with Other Testing Methodologies

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ABSTRACT

Background. The reference frozen-form broth microdilution (BMD) method for telavancin MIC determinations was optimized. This revised method now utilizes dimethyl sulfoxide as solvent and diluent for stock solution preparation and dilution for panel production, following the CLSI guidelines for water-insoluble agents. The revised BMD method also adds polysorbate-80 (P-80; or Tween-80; 0.002%) in the BMD test medium. Like dalbavancin and oritavancin, addition of P-80 was deemed necessary for more accurate and reproducible telavancin MIC determinations by minimizing drug binding to the 96-well plastic plates. This study evaluated the impact of the revised method on telavancin MIC results when compared with a previously established CLSI method. The performance of a new commercial dry-form panel formulation was also assessed.
Materials. 462 wildtype and a challenge set of Gram-positive isolates were simultaneously tested using the revised method, the previous method, and a newly developed dry-form panel formulation. All panels were manufactured by TREK Diagnostics. Isolates were tested using CLSI methods and MIC results were quality assured using ATCC QC strains. Telavancin MIC values obtained by the revised BMD method were considered as reference results. MIC values obtained by the dry-form panel that were within ± one log_2 dilution step when compared to the revised BMD results were considered as essential agreement (EA) and acceptable.
Results. Overall, 71.6% of telavancin MIC results obtained by the revised method were one or two doubling dilutions lower than the previously established method results. MIC_{50} values for staphylococci and enterococci obtained by the revised BMD were eight- to four-fold lower than those obtained by the previous method. The MIC_{50} results for streptococci obtained by the revised method were two- to four-fold lower than those from the previous method. 98.7% of the telavancin MIC results obtained by the new dry-form panel formulation were equivalent (± one log_2 dilution step) to those generated by the revised method. High EA (≥99%) between the dry-form and revised BMD MIC results were obtained across each species, except against the challenge set (96.4%) and *S. pneumoniae* (94.0%) organisms.
Conclusion. Similar to other lipoglycopeptides, telavancin MIC values should be determined using the revised BMD method. The revised BMD method can be utilized in the clinical microbiology laboratories along with associated MIC QC ranges and newly updated interpretive breakpoints established by the Food and Drug Administration (FDA).

INTRODUCTION

- Telavancin is a lipoglycopeptide antimicrobial agent with potent bactericidal activity *in vitro* against Gram-positive bacteria including methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA), heterogeneous (h)VISA, and multidrug-resistant streptococci and enterococci.[1]
- The *in vitro* antimicrobial activity of telavancin reported to date has been determined using the previously established broth microdilution (BMD) susceptibility testing method.[2]
  - This method consists of the use of dimethyl sulfoxide (DMSO) as solvent for stock solution preparation, and water as stock solution diluent for manufacturing 96-well frozen-form panels according to the Clinical and Laboratory Standards Institute (CLSI) recommendations described in the M100-S23 (2013) and previous documents.[2]
- However, during the development of dalbavancin and oritavancin, both lipoglycopeptide agents, the use of the surfactant polysorbate-80 (P-80; 0.002% final testing concentration) was shown to be essential for accurate minimum inhibitory concentration (MIC) susceptibility testing determinations.[3,4]
- Surfactants, such as P-80, act as wetting agents and are commonly used in commercially prepared antimicrobial agent susceptibility testing panels, or as part of the inoculum for BMD assays to aid in the homogenous dispersal of reagents or to ensure their quantitative recovery from solution.[3,4]
- The antimicrobial susceptibility testing for these lipoglycopeptide agents were revised, and updated quality control (QC) ranges for dalbavancin and oritavancin were established and published in the CLSI guidance documents.[5,6]
- These precedents led to the evaluation of P-80 on telavancin BMD MIC testing. Changes in solvents and/or diluents to achieve optimal drug solubilization were also simultaneously addressed.
- Further investigations proposed the use of DMSO as both solvent and diluent (instead of water as diluent) for telavancin stock solution preparation and dilution for MIC panel manufacturing. P-80 was also incorporated to the test media.
- These changes were shown to improve the drug solubility during panel preparation (DMSO for stock solution preparation and dilution) and drug availability in the 96-well plastic plates (P-80).
- Initial studies using this revised method demonstrated that the MIC_{50} results for telavancin were four- to eight-fold lower than those obtained by the previously established method when tested against staphylococci and enterococci, but minimal differences were observed when testing streptococci (data on file; JMI Laboratories).
- Thus, the purpose of this study was to fully evaluate telavancin MIC results when using a revised BMD method compared with those obtained by the previously established CLSI method when tested against a larger collection of clinically relevant strains.
- In addition, the telavancin MIC results obtained with the revised BMD method were compared with several candidate dry-form formulation panels.

MATERIALS AND METHODS

Clinical and reference isolates

- A total of 462 clinical isolates were included in this study.
- Initially, Gram-positive clinical strains collected during previous world-wide surveillance programs (89.6% from the 2009 surveyed year) were selected.
- These strains originated predominantly from hospitals in the USA (51.7%) and Europe (47.8%), and included: *S. aureus* (100 strains), coagulase-negative staphylococci (CoNS; 101 strains), *Enterococcus faecalis* (61; 15 VanA, five VanB resistance phenotypes, and 41 vancomycin-susceptible strains), *Enterococcus faecium* (44; 17 VanA, six VanB resistance phenotypes and 21 vancomycin-susceptible strains), *Streptococcus pneumoniae* (50 strains), viridans group streptococci (VGS; 25 strains), and ß-hemolytic streptococci (ßHS; 25 strains).
- Secondly, a challenge set of organisms (56 strains) displaying several key antimicrobial susceptibility phenotypes were selected and included in this study as follows: hVISA (11 strains), VISA (five strains), vancomycin-resistant *S. aureus* (VRSA; six strains), vancomycin-resistant enterococci (VRE; four *E. faecalis* [two VanA- and two VanB-types] and six *E. faecium* [four VanA- and two VanB-types]), daptomycin-non-susceptible staphylococci (six *S. aureus* and seven CoNS), and linezolid-resistant staphylococci (four *S. aureus* and seven *S. epidermidis*). Some of the isolates included in this set (22 strains) were provided by the Network on Antimicrobial Resistance in *S. aureus* (NARSA, www.narsa.net).

Antimicrobial susceptibility testing

- Telavancin stock solutions were dissolved and diluted in DMSO following the CLSI (Table 8B; M100-S24, 2014) recommendations for water-insoluble agents for the preparation of frozen-form panels according to the revised method.[7]
- Frozen-form panels produced according to the previously established susceptibility testing method were manufactured following the previous CLSI recommendations (M100-S23). Several Sensititre™ dry-form BMD panel candidate formulations (eight) were manufactured and tested simultaneously with the previously established and revised frozen-form panels.[2]
- All 96-well panels were manufactured by ThermoFisher Scientific (formerly TREK Diagnostics Systems/Sensititre™; Cleveland, OH, USA), following the recommendations described in the M07-A9 document. Mueller-Hinton broth (MHB) was supplemented with 2.5–5% lysed horse blood (LHB) for testing fastidious streptococci.[8]
- Telavancin MIC ranges when tested against American Type Culture Collection (ATCC) strains using the revised BMD method were those established during a QC study conducted according to the CLSI M23-A3 (2008) guideline document.
- The MIC QC ranges for telavancin, when applying the revised BMD method, are available in the recently published M100-S24 document, as follows: *S. aureus* ATCC 29213, 0.03–0.12 µg/mL; *E. faecalis* ATCC 29212, 0.03–0.12 µg/mL; and *S. pneumoniae* ATCC 49619, 0.004–0.015 µg/mL (see poster #2566 for additional information).[7]
- Telavancin MIC ranges when tested against ATCC strains using the previously established BMD method, were those published in the M100-S23 and previous documents.[2]
- All telavancin MIC QC values obtained by frozen-form panels prepared according to the previously established and revised methods were within the ranges published in the M100-S23 and M100-S24 documents, respectively.[2,7]
- Telavancin MIC values obtained by the revised BMD method were considered as reference results for these analyses.
- MIC values obtained by the previously established frozen-form and dry-form formulation panels that were between ± one log_2 dilution step when compared to the revised method were considered as essential agreement (EA).
- The minimal acceptable criteria for EA was targeted at ≥90%.[9]

RESULTS

- Overall, the majority (345/462, 74.7%) of telavancin MIC results obtained by the previously established method were ≥two log_2 dilution steps higher than the revised BMD method, which translated into low EA between the two methods (**Table 1**).
- When telavancin was tested using the previously established BMD method, >96.0% of *S. aureus* and CoNS clinical isolates had telavancin MIC results two to three doubling dilutions higher than those obtained by the revised method (**Table 1**). These lower results obtained by the revised BMD method translated into telavancin modal MIC and MIC_{50} values of 0.03 and 0.06 µg/mL for *S. aureus* and CoNS, respectively, which were eight- and four-fold lower than those obtained by the previously established BMD method (all 0.25 µg/mL) (**Table 2**).
- Similarly, *E. faecalis* and *E. faecium*, when tested by the previously established method, had most MIC results two log_2 dilutions higher than those obtained by the revised BMD method (**Table 1**). The previously established method generated MIC_{50} results against *E. faecalis* (MIC_{50}, 0.5 µg/mL) and *E. faecium* (MIC_{50}, 0.25 µg/mL) four- and eight-fold higher than the revised method (MIC_{50} values of 0.12 and 0.03 µg/mL, respectively) (**Table 2**).
- Differences in MIC results between frozen-form BMD methods were less significant for the streptococci, where the majority of MIC values obtained by the previously established method were only one doubling dilution step higher than the revised method (**Table 1**).
- This observation translated into greater equivalence between methods for both ßHS and VGS, which exhibited telavancin MIC_{50} values of 0.06 µg/mL by the previously established method, while two-fold lower MIC_{50} results were noted by the revised method (ie 0.03 µg/mL).
- The previously established method produced most MIC results against *S. pneumoniae* that were one (50.0%; 25/50) or two (40.0%; 20/50) log_2 dilutions higher than those obtained by the revised method, with final MIC_{50} results by the former (0.03 µg/mL) four-fold higher than the latter (0.008 µg/mL) (**Table 1** and **Table 2**).
- Among candidate dry-form panels tested, one formulation had highest overall EA rates (98.7%) when compared to the revised method (**Table 3**). EA rates of ≥99.0% were observed for all species or group of organisms, except for *S. pneumoniae* (94.0%) and the challenge set (96.4%), but all above target EA (≥90.0%).

Table 1. MIC result variations and summary of essential agreement rates between a previously established BMD method and the new revised BMD method for telavancin

Organism (no. tested)	Log_2 MIC variations compared to the revised BMD[a]						% EA[b]
	-2	-1	0	+1	+2	≥+3	
S. aureus (100)	0	0	0	3	44	53	3.0
CoNS (101)	0	0	0	4	77	20	4.0
E. faecalis (61)[c]	0	0	7	12	26	16	31.1
E. faecium (44)[d]	0	0	3	14	19	8	38.6
S. pneumoniae (50)	0	1	3	25	20	1	58.0
ßHS (25)	0	0	1	13	11	0	56.0
VGS (25)	0	0	0	18	6	1	72.0
Challenge (56)[e]	0	1	1	11	27	16	23.2
All (462)	0	2	15	100	230	115	25.3

[a] Previously established BMD panels prepared with DMSO as solvent and water as diluent, and no P-80 supplementation versus a revised BMD panel (DMSO as solvent and diluent, and P-80 supplementation [0.002%]).
[b] Percentage of EA (± one log_2 dilution step).
[c] Includes 20 VRE (15 VanA and five VanB phenotypes).
[d] Includes 23 VRE (17 VanA and six VanB phenotypes).
[e] Represent strains with key resistance phenotypes (11 hVISA, five VISA, six VRSA, 10 VRE, 13 daptomycin-nonsusceptible staphylococci, 11 linezolid-resistant staphylococci).
ßHS = ß-hemolytic streptococci; BMD = broth microdilution; CoNS = coagulase-negative staphylococci; DMSO = dimethyl sulfoxide; EA = essential agreement; MIC = minimum inhibitory concentration; VGS = viridans group streptococci; VRE = vancomycin-resistant enterococci.

Table 2. *In vitro* MIC results for telavancin when tested against Gram-positive isolates using a previously established BMD method and the new revised BMD method

Organism (no. tested)	Method[a]	MIC (µg/mL)			
		Range	Mode	50%	90%
All (462)	Previous	≤0.004–>8	0.25	0.25	2
	Revised	≤0.004–8	0.03	0.06	0.25
S. aureus (100)	Previous	0.06–0.5	0.25	0.25	0.5
	Revised	0.015–0.25	0.03	0.03	0.06
CoNS (101)	Previous	0.06–0.5	0.25	0.25	0.5
	Revised	0.015–0.12	0.06	0.06	0.06
E. faecalis[b] (61)	Previous	0.12–>8	0.5	0.5	8
	Revised	0.03–8	0.12	0.12	4
E. faecalis VanS (41)	Previous	0.12–1	0.5	0.5	1
	Revised	0.03–0.25	0.12	0.12	0.12
E. faecium[c] (44)	Previous	0.06–4	0.12, 4[d]	0.25	4
	Revised	0.015–4	0.03	0.03	2
E. faecium VanS (21)	Previous	0.06–0.5	0.12	0.12	0.25
	Revised	0.015–0.06	0.03	0.03	0.03
S. pneumoniae (50)	Previous	≤0.004–0.12	0.03	0.03	0.06
	Revised	≤0.004–0.06	0.008	0.008	0.03
ßHS (25)	Previous	0.03–0.12	0.06	0.06	0.12
	Revised	0.015–0.06	0.03	0.03	0.03
VGS (25)	Previous	0.03–0.12	0.06	0.06	0.12
	Revised	0.015–0.06	0.03	0.03	0.03
Challenge (56)[e]	Previous	0.06–8	0.25, 0.5[d]	0.5	4
	Revised	0.015–8	0.06	0.06	4

[a] Previously established BMD panels prepared with DMSO as solvent and water as diluent, and no P-80 supplementation versus a revised BMD panel (DMSO as solvent and diluent, and P-80 supplementation (0.002%)).
[b] Includes 20 VRE (15 VanA and five VanB phenotypes).
[c] Includes 23 VRE (17 VanA and six VanB phenotypes).
[d] Bimodal MIC distribution (two modal values).
[e] Represent strains with key resistance phenotypes (11 hVISA, five VISA, six VRSA, 10 VRE, 13 daptomycin-nonsusceptible staphylococci, 11 linezolid-resistant staphylococci).
ßHS = ß-hemolytic streptococci; BMD = broth microdilution; CoNS = coagulase-negative staphylococci; DMSO = dimethyl sulfoxide; MIC = minimum inhibitory concentration; VanS = vancomycin-susceptible; VGS = viridans group streptococci; VRE = vancomycin-resistant enterococci.

Table 3. MIC result variations and summary of essential agreement rates between a dry-form BMD formulation panel (Sensititre™) and the revised BMD method for telavancin

Organism (no. tested)	Log_2 variations compared to the revised BMD[a]					% EA[b]
	-2	-1	0	+1	+2	
S. aureus (100)	1	5	57	37	0	99.0
CoNS (101)	0	24	70	7	0	100.0
E. faecalis (61)[c]	0	12	45	4	0	100.0
E. faecium (44)[d]	0	9	33	2	0	100.0
S. pneumoniae (50)	3	7	27	13	0	94.0
ßHS (25)	0	9	12	4	0	100.0
VGS (25)	0	8	16	1	0	100.0
Challenge (56)[e]	2	13	36	4	0	96.4
All (462)	6	87	296	73	0	98.7

[a] Previously established BMD panels prepared with DMSO as solvent and water as diluent, and no P-80 supplementation versus a revised BMD panel (DMSO as solvent and diluent, and P-80 supplementation (0.002%)).
[b] Percentage of EA (± one log_2 dilution step).
[c] Includes 20 VRE (15 VanA and five VanB phenotypes).
[d] Includes 23 VRE (17 VanA and six VanB phenotypes).
[e] Represent strains with key resistance phenotypes (11 hVISA, five VISA, six VRSA, 10 VRE, 13 daptomycin-nonsusceptible staphylococci, 11 linezolid-resistant staphylococci).
ßHS = ß-hemolytic streptococci; BMD = broth microdilution; CoNS = coagulase-negative staphylococci; DMSO = dimethyl sulfoxide; EA = essential agreement; MIC = minimum inhibitory concentration; VGS = viridans group streptococci; VRE = vancomycin-resistant enterococci.

CONCLUSIONS

- The revised BMD method was previously shown to improve the drug solubility during panel preparation (DMSO for stock solution preparation and dilution) and drug availability in the 96-well plastic plates (P-80) and, most importantly, to provide a more accurate and reliable MIC determination for telavancin.
- The data presented here show that the revised BMD method results in lower MIC values when compared with those obtained by the previously established method, especially when tested against staphylococci and enterococci. This revised method is now consistent with those utilized for other lipoglycopeptides (dalbavancin and oritavancin).[3,4]
- The impact of the revised method on the telavancin MIC results when tested against streptococci was less pronounced, which was also similar to that observed for the other lipoglycopeptides. This result suggests that the presence of LHB provides an effect similar to that of P-80.[3,4]
- The results presented here also validate a commercial dry-form formulation panel, which can be used as an alternative method for telavancin susceptibility testing in the clinical microbiology setting, after appropriate regulatory approvals (together with adequate QC ranges and interpretive breakpoints).

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