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Assessment of telavancin minimal inhibitory concentrations by revised broth microdilution method in phase 3 complicated skin and skin-structure infection clinical trial isolates

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ABSTRACT

The broth microdilution (BMD) MIC testing method for telavancin was recently revised BMD (rBMD) to improve accuracy and reproducibility. *Staphylococcus aureus* isolates from telavancin phase 3 complicated skin and skin-structure infection (cSSSI) studies were tested using the rBMD method. Retesting of 1132 isolates produced MICs ranging from ≤0.015 to 0.12 μg/mL that were 8-fold lower than the original method. All isolates tested remained susceptible to telavancin at the revised susceptibility breakpoint of 0.12 μg/mL. The clinical cure and microbiological eradication rates were 90% (368/409) and 89% (366/409) for telavancin-treated patients, and were similar for patients with methicillin-susceptible and -resistant *S. aureus* isolates and *S. aureus* isolates with elevated vancomycin MICs (≥1 μg/mL). The data presented here are aimed to update the literature and better inform clinicians and clinical microbiologists about the revised telavancin MICs, as well as the corresponding clinical and microbiological cure rates for cSSSI patients.

1. Introduction

Telavancin (VIBATIV®, Theravance Biopharma Antibiotics, Inc. George Town, Grand Cayman, Cayman Islands) is an US Food and Drug Administration (FDA) approved, commercially available, once-daily, parenterally administered lipoglycopeptide antibacterial agent that has demonstrated activity against numerous pathogenic Gram-positive bacteria, including methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), heterogeneous vancomycin-intermediate *S. aureus*, vancomycin-intermediate *S. aureus*, and organisms nonsusceptible to other agents, such as linezolid and daptomycin (King et al., 2004; Leuthner et al., 2006; Pace et al., 2003; Smith et al., 2015; VIBATIV® [package insert], 2016). The safety and clinical efficacy of telavancin have been previously evaluated in the Assessment of Telavancin in Complicated Skin and Skin-Structure Infections (cSSSI) studies (ATLAS) (Stryjewski et al., 2008).

The broth microdilution (BMD) MIC testing method for telavancin was revised and optimized (revised BMD [rBMD]) in 2014 to provide more accurate, precise, and reproducible MIC results (Clinical and Laboratory Standards Institute, 2014). The reasons for the revision were to both improve the solubility of telavancin by including dimethyl sulfoxide (DMSO) during preparation and to minimize telavancin binding to plastic surfaces by adding polysorbate 80 (P-80) to the cation adjusted Mueller-Hinton broth (Arhin et al., 2008; Fritsche et al., 2006). The Clinical Laboratory Standards Institute (CLSI)- and FDA-approved telavancin susceptibility breakpoint for *S. aureus*, for both MSSA and MRSA isolates, is ≤0.12 μg/mL due to the rarity of telavancin-resistant *S. aureus* isolates (Clinical and Laboratory Standards Institute, 2016; VIBATIV® [package insert], 2016).

The objective of this study was to analyze the *S. aureus* isolates recovered from patients treated in the ATLAS studies using the rBMD method and evaluate the impact of the revised MICs on the clinical cure and microbiological eradication rates. These results were part of the data package presented to breakpoint setting committees, including FDA, CLSI, and the European Committee on Antimicrobial Susceptibility Testing for the evaluation of the revised telavancin breakpoints. This manuscript is intended to inform clinicians and clinical microbiologists regarding the revised in vitro telavancin breakpoints and MIC in cSSSI patients.

2. Methods

2.1. Patient population and study procedures

The protocol for the ATLAS studies has been described elsewhere in detail (Stryjewski et al., 2008) (ClinicalTrials.gov identifiers NCT00091819 and NCT00107978). Briefly, the ATLAS studies were 2

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phase 3, multicenter, randomized, double-blind, parallel-group, active-control studies conducted in parallel, which included a prespecified plan for pooled analysis design. Patients ≥18 years old with cSSSI caused by suspected or confirmed Gram-positive organisms were randomly assigned to treatment with either telavancin (10 mg/kg every 24 hours) or vancomycin (1 g every 12 hours). The primary end point of the study was the investigator-assessed clinical response at the test-of-cure visit between 7 and 14 days after the last dose of study medication. Primary infection site and blood culture specimens were collected at baseline, and isolates underwent susceptibility testing and confirmation at a central laboratory (Covance Laboratories, Indianapolis, IN, United States). In these studies, the efficacy of telavancin was noninferior to that of vancomycin, and the safety profile of telavancin in the studies was considered acceptable for the treatment of patients with serious cSSSI (Stryjewski et al., 2008).

Clinical cure rates by MIC reported in the ATLAS trials were reassessed with the MICs obtained using the rBMD method. In the ATLAS studies, the clinically evaluable study population consisted of patients who met study inclusion criteria, adhered to study protocol, and had a clinical response of either cure or failure at the test-of-cure visit. The microbiologically evaluable population in the ATLAS studies included all clinically evaluable patients who had a baseline Gram-positive (*S. aureus* in this analysis) pathogen retrieved from pretreatment cultures. In the microbiologically evaluable populations, the baseline pathogen was considered to be eradicated if it was not detected by culture or was presumed to be eradicated if the patient's clinical response was cure and there was no lesion from which a sample could be obtained for culture (Stryjewski et al., 2008).

2.2. Revised BMD method

The MIC analysis using the rBMD method was carried out at JMI Laboratories (North Liberty, IA, United States). Telavancin was solubilized and diluted in DMSO per CLSI recommendations for water-insoluble agents (Clinical and Laboratory Standards Institute, 2014). Briefly, the dry powder was dissolved in 100% DMSO in a glass vial to obtain an initial stock concentration of 1600 μg/mL. This stock solution was again diluted using 100% DMSO in accordance with the dilution scheme outlined in Table 8B of M100-S24 (Clinical and Laboratory Standards Institute, 2014). The telavancin-in-DMSO stock was further diluted (100×) in cation-adjusted Mueller-Hinton broth that was supplemented with the surfactant P-80 (tween, 0.002% *v*/v ratio) to minimize telavancin binding to plastic surfaces. Aliquots (100 μL) of the final telavancin concentrations (ranging from 0.004 μg/mL to 4 μg/mL) were dispensed into 96-well plates. MIC values for telavancin and control agents tested against *S. aureus* using the CLSI-approved and accepted quality control ranges (telavancin, 0.03–0.12 μg/mL; ampicillin, 0.5–2.0 μg/mL; linezolid, 1.0–4.0 μg/mL; vancomycin, 0.50–2.0 μg/mL).

2.3. Clinical isolates

All available microbiologically evaluable *S. aureus* isolates from the telavancin phase 3 ATLAS studies were tested using the rBMD method. These isolates were sent directly from the original central laboratory sites to JMI Laboratories. The isolates were stored at −80 °C and were shipped on dry ice. For the purposes of quality assurance, MIC results were validated using the CLSI-recommended American Type Culture Collection (ATCC®) quality control strain for *S. aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), and *Streptococcus pneumoniae* (ATCC 49619) (Ross et al., 2014).

3. Results

A total of 1132 microbiologically evaluable *S. aureus* isolates from the ATLAS studies were tested for telavancin MICs using the rBMD method, including 432 MSSA isolates and 700 MRSA isolates. One MSSA isolate that did not have an initial central laboratory MIC result was not included in the original susceptibility analysis but was included in this isolate set for rBMD testing. A unimodal distribution of MIC values was observed, ranging from ≤0.015 μg/mL to 0.12 μg/mL for all isolates (0.015–0.06 μg/mL for MSSA and 0.03–0.12 μg/mL for MRSA) with an MIC_{90} of 0.06 μg/mL for MSSA and MRSA subsets (Supplementary Fig. 1, Table 1). The results described with the rBMD method represent a downward shift in the *S. aureus* MICs by approximately 8-fold versus the original BMD method. With the original method, the distribution of MICs ranged from 0.12 μg/mL to 1.0 μg/mL and the MIC_{90} was 0.50 μg/mL (Supplementary Fig. 1) (Stryjewski et al., 2008). The previous susceptibility breakpoint based on the original BMD method was 1 μg/mL, and all isolates were susceptible to telavancin at the revised FDA approved breakpoint of 0.12 μg/mL. There were 849 isolates with elevated vancomycin MICs (≥1 μg/mL), for which telavancin MICs ranged from 0.03 μg/mL to 0.12 μg/mL, with $MIC_{50/90}$ values of 0.06/0.06 μg/mL (Table 1).

The retested vancomycin MIC range and $MIC_{50/90}$ values for all *S. aureus* isolates were 0.5 μg/mL to 2 μg/mL and 1/1 μg/mL, respectively (Supplementary Table 1). For the isolates with vancomycin MICs ≥1 μg/mL (n = 849) that were retested, the MIC values ranged from 1 μg/mL to 2 μg/mL, with $MIC_{50/90}$ values of 1/1 μg/mL (Supplementary Table 1). The rBMD method produced a similar shift in isolates with elevated vancomycin MICs compared with the original method. A total of 384 isolates exhibited a ±1 dilution change in the vancomycin MIC ≥1 μg/mL upon retesting. Of these, 224 increased from 0.5 μg/mL to 1 μg/mL, 2 increased from 1 μg/mL to 2 μg/mL, 10 decreased from 2 μg/mL to 1 μg/mL, and 148 decreased from 1 μg/mL to 0.5 μg/mL. Three isolates exhibited a 2-dilution change in the vancomycin MIC upon retesting, decreasing from 2.0 μg/mL to 0.5 μg/mL. These changes can be attributed to random testing differences between 2 laboratories.

Table 1
Telavancin MIC population distribution by rBMD method for retesting of *S. aureus* isolates from the ATLAS studies.

	Method	N	MIC (μg/mL)							Range (μg/mL)	MIC_{50}/MIC_{90} (μg/mL)
			0.015	0.03	0.06	0.12	0.25	0.5	1.0		
All *S. aureus* isolates	rBMD	1132	1	424	701	6				0.015–0.12	0.06/0.06
	Original	1131				25	474	574	58	0.12–1	0.5/0.5
MSSA	rBMD	432	1	200	231					0.015–0.06	0.06/0.06
	Original	431				20	189	204	18	0.12–1	0.5/0.5
MRSA	rBMD	700		224	470	6				0.03–0.12	0.06/0.06
	Original	700				5	285	370	40	0.12–1	0.5/0.5
All *S. aureus* vancomycin MIC ≥1 μg/mL	rBMD	849		221	622	6				0.03–0.12	0.06/0.06
	Original	776				13	423	300	40	0.12–1	0.25/0.5
MSSA vancomycin MIC ≥1 μg/mL	rBMD	289		91	198					0.03–0.06	0.06/0.06
	Original	254				9	148	86	11	0.12–1	0.25/0.5
MRSA vancomycin MIC ≥1 μg/mL	rBMD	560		130	424	6				0.03–0.12	0.06/0.06
	Original	522				4	275	214	29	0.12–1	0.25/0.5

MIC_{50} = lowest telavancin concentration required to inhibit 50% of isolates tested; MIC_{90} = lowest telavancin concentration required to inhibit 90% of isolates tested.
Data presented as number of isolates except where indicated.