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VISA–Daptomycin non-susceptible *Staphylococcus aureus* frequently demonstrate non-susceptibility to Telavancin

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ABSTRACT

Telavancin was evaluated against *S. aureus* isolates with reduced susceptibility to other antimicrobial agents using two broth microdilution methods and Etest® strips. The three methods provided comparable results. Differences in telavancin susceptibility versus non-susceptibility were noted mainly in the VISA-daptomycin non-susceptible group of isolates. In this group the percent susceptibility was 38% for the Etest® method and 50% and 54% for the 2 broth microdilution methods. All differences in susceptibility were within one 2-fold dilution.

1. Introduction

Telavancin is a lipoglycopeptide antibiotic that exhibits concentration dependent bactericidal activity via a dual mechanism of action involving inhibition of bacterial cell wall synthesis as well as disruption of cell membranes (Saravolatz et al., 2009). Telavancin has shown potent in vitro bactericidal activity against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-intermediate *Staphylococcus aureus* (VISA). Telavancin has been approved in the United States and Canada for the treatment of adult patients with complicated skin and skin structure infections and hospital-acquired and ventilator-associated bacterial pneumonias due to susceptible isolates of *S. aureus*. In the European Union, telavancin was approved for the treatment of nosocomial pneumonia, known or believed to be caused by methicillin-resistant *Staphylococcus aureus* (MRSA) when other alternative medicines are unsuitable. Due to the low usage of telavancin in the European Union (EU), the marketing authorization in the EU was withdrawn on March 23, 2018.

In January 2014, the Clinical and Laboratory Standards Institute (CLSI) published a revised broth microdilution susceptibility testing method for telavancin (CLSI, 2014). This method has also been approved by the Food and Drug Administration (FDA). (VIBATIV® Package Insert, 2016). The revised method follows the CLSI guidelines for water-insoluble agents and uses dimethyl sulfoxide as the solvent and diluent for antibiotic preparation. This method also includes the addition of polysorbate-80 at 0.002%. The polysorbate-80 is used to mitigate the propensity of the lipoglycopeptides to bind to plastics (Farrell et al., 2014). Because of these changes, the quality control ranges for *S. aureus* ATCC 29213 with telavancin and the telavancin susceptibility breakpoints have also changed. The previously acceptable quality control ranges for ATCC 29213 of 0.12–1 μg/mL have changed to 0.03–0.12 μg/mL. The FDA susceptibility breakpoint of ≤1 μg/mL for *S. aureus* changed to ≤0.12 μg/mL.

The purpose of this study was to assess the activity of telavancin against a collection of *S. aureus* isolates with reduced susceptibility to agents including daptomycin and vancomycin using updated methodology with two approved broth microdilution methods and Etest® strips and to evaluate concordance between these methods.

2. Methods

A collection of 74 isolates were used for in vitro testing. Vancomycin and daptomycin susceptible *Staphylococcus aureus* ($n = 16$), vancomycin susceptible and daptomycin non-susceptible *S. aureus* ($n = 9$), daptomycin susceptible and vancomycin intermediate *Staphylococcus aureus* (VISA) ($n = 8$), daptomycin non-susceptible VISA ($n = 26$), and vancomycin resistant *Staphylococcus aureus* (VRSA) ($n = 15$). Strains were obtained from patients admitted to St. John Hospital and Medical Center (Detroit, MI) and the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program (this program is now referred to as Biodefense and Emerging Infections Research Resources Repository [BEI Resources]).

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Three different methods were used to determine the telavancin MIC: reference broth microtiter plates prepared in house (BMD), lyophilized dry format Sensititre® Custom plates (CMP4STA) (ThermoFisher) and Etest® strips (BIOMERIEUX). The Sensititre® plates contained telavancin, vancomycin, teicoplanin, oxacillin, daptomycin, linezolid, quinupristin-dalfopristin, clindamycin, ciprofloxacin, tigecycline, erythromycin, gentamicin and trimethoprim-sulfamethoxazole. All plates were inoculated with approximately 5×10^5 CFU/ml of each isolate and the inoculum was verified by colony counts. A new bacterial suspension was prepared for each testing method. All plates were incubated at 35 °C for 18 to 24 hours in ambient air. MICs were read visually as the lowest drug concentration well with no visible bacterial growth. The testing with Etest® strips was performed per manufacturer's recommendations. Telavancin powder for the BMD, Sensititre® plates and the Etest® strips were provided by Theravance Biopharma Antibiotics, Inc. *Staphylococcus aureus* ATCC 29213 was used to monitor quality control for the agents. All testing procedures were performed in triplicate in accordance with CLSI guidelines and the mode of the results was used to report the end results (CLSI, 2017). Susceptibility categories were determined per CLSI breakpoints.

3. Results

Table 1 compares the telavancin results from the three different testing methods. The results from the isolates were categorized into 5 groups: 1) vancomycin and daptomycin susceptible (VS-DS), 2) vancomycin susceptible and daptomycin non-susceptible (VS-DNS), 3) vancomycin intermediate and daptomycin susceptible (VISA-DS), 4) vancomycin intermediate and daptomycin non-susceptible (VISA-DNS), and 5) VRSA. The VRSA isolates were all daptomycin susceptible.

For the VS-DS, VS-DNS and VRSA groups the percent susceptible within each group was the same when comparing all three testing methods. In the VISA-DS group, the percent susceptible was 100% with the Sensititre® plate and 88% with the BMD and.

E-test® procedure. This difference in susceptibility was caused by one isolate. In the VISA-DNS group, percent susceptible ranged from 38% to 54%; these differences in susceptibility were caused by nine isolates. All these differences were within one doubling dilution as can be seen in Fig. 1. Fig. 1 contains scatter grams which plots and compares the telavancin MIC values from the different methods. Fig. 1 also displays the susceptibility variation and the discordant results between each of the three testing methods.

4. Discussion

Many of the previous telavancin studies utilizing the revised testing methods have shown 100% susceptibility when testing telavancin against *S. aureus* isolates (Duncan et al., 2016; Jones et al., 2017; Mendes et al., 2015, 2017; Smart et al., 2017). As seen in Table 1, we have reported a lower rate of telavancin susceptibility. Our study is not a prevalence study and our results are a consequence of use of a challenge set of *S. aureus* isolates. All the VS-DS and VS-DNS were susceptible to telavancin, and telavancin non-susceptibility was only seen in the VISA and VRSA isolates. The VRSA isolates all demonstrated telavancin non-susceptibility; previous studies have also reported telavancin's lack of activity against VRSA isolates (Farrell et al., 2014; Karlowsky et al., 2015; Saravolatz et al., 2012).

The considerable amount of telavancin non-susceptibility among MRSA isolates that are both vancomycin intermediate and daptomycin non-susceptible seen in our study has not been previously reported. We have found other studies that tested VISA isolates and DNS isolates, but we have been unable to find reports that tested as many isolates as we studied that were both VISA and DNS (Karlowsky et al., 2015; Mendes et al., 2015, 2017; Smith et al., 2015). In previous studies, it has been observed that the telavancin MIC results increase when the isolates have a higher vancomycin or daptomycin MIC (Duncan et al., 2016; Mendes et al., 2015, 2017). This phenomenon may be exaggerated when the isolates have both an increased vancomycin and daptomycin MIC. As clinicians are much more likely to encounter, daptomycin non-susceptible VISA infections then VRSA infections, they should be aware that telavancin may also be ineffective in many of these cases. It is doubtful that this observation is due to a clonal phenomenon, as isolates tested within the VISA–DNS phenotype had five different genetic groups when reviewing their staphylococcal cassette chromosome *mec* typing and the multi-locus sequence typing performed by NARSA.

Overall, our results showed good concordance between the three testing methods, with each method being a reliable method to test telavancin susceptibility. All three methods demonstrated similar in vitro activity for isolates that are vancomycin susceptible and VS-DNS. All three methods consistently identified the vancomycin resistant strains as telavancin non-susceptible. Although the MIC_{50} and MIC_{90} for all three methods were similar, the actual telavancin susceptibility was lower when tests were performed for isolates that were vancomycin intermediate and daptomycin non-susceptible. Clinicians should be aware that all three methods will generally provide comparable results for telavancin susceptibility testing except for VISA-daptomycin non-susceptible strains. If one encounters infections due to *S. aureus* that are both daptomycin non-susceptible and vancomycin intermediate, repeat susceptibility testing by a different method should be considered to assure telavancin susceptibility before the clinician commits to telavancin therapy in these infections.

Table 1
Telavancin susceptibility testing results by three methods.

							Broth Microtiter Plates TLV Results				Sensititre® Plates TLV Results				E-test Strip® TLV Results			
VAN MIC Range µg/mL	VAN MIC_{50}	VAN MIC_{90}	DAP MIC Range µg/mL	DAP MIC_{50}	DAP MIC_{90}	N	Range µg/mL	MIC_{50} µg/mL	MIC_{90} µg/mL	TLV % Sus	Range µg/mL	MIC_{50} µg/mL	MIC_{90} µg/mL	TLV %Sus	Range µg/mL	MIC_{50} µg/mL	MIC_{90} µg/mL	TLV %Sus
0.1–2.0 (**S**)	2	2	0.5–1.0 (**S**)	0.5	1	16	0.06–0.12	0.06	0.06	100%	0.03–0.12	0.06	0.12	100%	0.032–0.125	0.047	0.094	100%
2 (**S**)	2	NC	2.0- > 4.0 (**NS**)	2	NC	9	0.06–0.12	0.12	NC	100%	0.06–0.12	0.12	NC	100%	0.047–0.125	0.064	NC	100%
4.0–8.0 (**IN**)	4	NC	0.5–1.0 (**S**)	1	NC	8	0.03–0.25	0.12	NC	88%	0.06–0.12	0.12	NC	100%	0.047–0.19	0.125	NC	88%
4.0–8.0 (**IN**)	4	8	2.0- > 4.0 (**NS**)	2	4	26	0.06–0.5	0.12	0.25	54%	0.06–0.5	0.12	0.25	50%	0.064–0.5	0.19	0.38	38%
> 32 (**R**)	>32	>32	0.25–1.0 (**S**)	0.5	1	15	0.25–4	2	4	0%	0.5–4	2	4	0%	0.25–4	1	4	0%

S=Susceptible.
NS=NonSusceptible.
IN=Intermediate.
R = Resistant.
NC=Not Calculated for N < 10.

A

E-Test Results for TLV MIC (µg/mL)	0.03	0.06	0.12	0.25	0.5	1	2	4
4								2
3								3
2							1	
1.5							1	
1					1	4	1	
0.75						1		
0.5					1			
0.38				2	1			
0.25			1	6				
0.19			4	3				
0.125			15	1				
0.094		2	4					
0.064		6	3					
0.047	1	5						
0.032	3	2						

Broth Microdilution Results for TLV MIC (µmL)

B

E-test Results for TLV MIC (µg/mL)	0.03	0.06	0.12	0.25	0.5	1	2	4
4							1	1
3							2	1
2							1	
1.5							1	
1					1	4	1	
0.75						1		
0.5					1			
0.38				3				
0.25			1	5	1			
0.19			4	3				
0.125		1	14	1				
0.094		2	4					
0.064	1	3	5					
0.047		5	1					
0.032		4	1					

Sensititre® Results for TLV MIC (µg/mL)

C

Sensititre® Results for TLV MIC (µg/mL)	0.03	0.06	0.12	0.25	0.5	1	2	4
4								2
2							3	3
1						5		
0.5				1	2			
0.25			4	7	1			
0.12		5	21	4				
0.06	4	9	2					
0.03		1						

Broth Microdilution Results for TLV MIC (µg/mL)

Fig. 1. A: Scattergram comparing the telavancin MIC values using E-test® versus broth microdilution plates for all isolates tested. The thick lines represent the breakpoint for each method. B: Scattergram comparing the telavancin MIC values by E-test® versus Sensititre® plates for all isolates tested. The thick lines represent the breakpoint for each method. C: Scattergram comparing the telavancin MIC values by Sensititre® plates versus broth microdilution plates for all isolated tested. The thick lines represent the breakpoint for each method.

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