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A Multi-Site Study Comparing an 18-24h Sensititre® Susceptibility System to the CLSI Broth Microdilution Method for Telavancin Using Fastidious and Non-Fastidious Gram-Positive Organisms

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Abstract

Background: : Telavancin (TLV) (Theravance, Inc., South San Francisco, CA) is a bactericidal lipoglycopeptide under investigation for the treatment of cSSSI and HAP caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and other Gram-positive bacteria. An evaluation was performed to determine the accuracy and reproducibility of TLV susceptibility testing using the Sensititre® 18-24h dried susceptibility system (TREK Diagnostic Systems, Cleveland, OH) compared with the CLSI M07 reference broth microdilution method (BMD). Both automated and manual reading methods were performed.

Methods: TLV (0.001-16 µg/mL) was tested against 897 recent clinical isolates, 150 challenge isolates and 50 reproducibility isolates. These isolates consisted of: 137 CNS spp., 186 *Staphylococcus aureus*, 128 *Enterococcus* spp., 326 *Streptococcus* spp. Beta-hemolytic grp., 186 *Streptococcus pneumoniae*, and 134 *Streptococcus* spp. Viridans grp. Dried plates were inoculated as per manufacturers' instructions and BMD was performed per CLSI M07. Recommended CLSI quality control (QC) organisms were tested daily and all results were within the CLSI published QC ranges.

Results: Comparisons of TLV MIC results on the Sensititre® system to the CLSI M07 BMD for both automated and manual reads resulted in 99.0% and 99.6% essential agreement (+/- one $\log_2$ dilution), respectively. Overall agreement for the reproducibility (+/- one $\log_2$ dilution of the modal MIC) for automated and manual reads were 99.5% and 98.5%, respectively.

Conclusions: The results for TLV indicate that the Sensititre® 18-24h susceptibility system for all clinical and challenge isolates gives reliable results using either the automated or manual read methods compared to the reference CLSI BMD.

Introduction

Telavancin is a bacteriacidal lipoglycopeptide antibiotic structure derived from vancomycin. It is highly active against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). Here we are reporting results from a multi-site study, performing a series of evaluations to determine the accuracy and reproducibility of the Sensititre® 18 – 24h susceptibility system with **telavancin** compared to the CLSI reference broth microdilution method (M07).

Materials & Methods

• Indications for use: The Sensititre® 18 – 24 hour MIC or breakpoint susceptibility system is an *in vitro* diagnostic product for clinical susceptibility testing of both fastidious and non-fastidious organisms.

• Each isolate was tested using a Sensititre® 18 – 24 hour susceptibility plate containing **telavancin** (0.001-16µg/mL) (Theravance, Inc., South San Francisco, CA). The dried plates were set-up and tested according to the manufacturers' instructions.

• The CLSI reference broth microdilution plate was prepared and tested on each isolate according to the Clinical Laboratory Standards Institute (CLSI M07).

• Testing consisted of 897 fresh clinical isolates (conducted at three different sites) including approximately 447 Gram-positive isolates and 450 fastidious isolates from each site. Also included were 75 Gram-positive and 75 fastidious challenge isolates from the Center for Disease Control and Prevention (CDC) that were supplied to a single testing site (Tables 1 and 2).

• Reproducibility testing consisted of 25 Gram-positive and 25 *Streptococcus* spp. isolates tested at all 3 sites on the Sensititre® 18 – 24 hour susceptibility plate (Table 1). The test plate results were compared with those of the CLSI reference broth microdilution plate.

• Quality control (QC) was assured by testing 20 replicates of each ATCC strain including *S. aureus* 29213, *E. faecalis* 29212, and *S. pneumoniae* 49619, at each of the three sites (Tables 1 and 3).

• Colony counts were performed on the inoculum of the QC strains on each day of testing.

Table 1. Organisms Tested

Organisms Tested	Number Tested
Clinical Isolates (3 sites)	
(447 Gram-positive, 450 *Streptococcus* spp.)	897
CDC Challenge Isolates (one site)	
(75 Gram-positive, 75 *Streptococcus* spp.)	150
Reproducibility Isolates (3 sites)	
(25 Gram-positive, 25 *Streptococcus* spp.)	50
CLSI Quality Control Strains	
(20 replicates of each strain at 3 sites)	3 x 20

Table 2. Clinical and Challenge Isolates Tested

Gram-positive Organisms	Number Tested
Coagulase Negative *Staphylococcus*	177
Staphylococcus aureus	121
Enterococcus spp.	130
Beta Streptococcus (MHB)	90
Total	**518**
***Streptococcus* spp.**	Number Tested
Streptococcus pneumoniae	176
Viridans Group *Streptococcus*	130
Beta Streptococcus (MHB with LHB)	220
Total	**526**

Table 3. Quality Control Strains

Quality Control Strains	CLSI MIC Ranges (µg/ml)
Staphylococcus aureus ATCC 29213	0.12-1
Enterococcus faecalis ATCC 29212	0.12-0.5
Streptococcus pneumoniae ATCC 49619	0.004-0.03

Results

Table 4. Summary Data and % Essential Agreement of Gram-positive Clinical and Challenge Isolates Using the Manual Read Method

Clinical Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	113	112	113	112	100.0%	100.0%
Staphylococcus aureus	150	150	150	150	100.0%	100.0%
Enterococcus spp.	105	101	103	99	98.1%	98.0%
Streptococcus spp. Beta-hemolytic Group with MHB	79	50	77	48	97.5%	96.0%
Total	**447**	**413**	**443**	**409**	**99.1%**	**99.0%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	17	17	17	17	100.0%	100.0%
Staphylococcus aureus	27	27	27	27	100.0%	100.0%
Enterococcus spp.	16	15	16	15	100.0%	100.0%
Streptococcus spp. Beta-hemolytic Group with MHB	11	6	11	6	100%	100%
Total	**71**	**65**	**71**	**65**	**100%**	**100%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	130	129	130	129	100.0%	100.0%
Staphylococcus aureus	177	177	177	177	100.0%	100.0%
Enterococcus spp.	121	116	121	116	100.0%	100.0%
Streptococcus spp. Beta-hemolytic Group with MHB	90	56	88	54	97.8%	96.4%
Total	**518**	**478**	**516**	**476**	**99.6%**	**99.6%**

Table 5. Summary Data and % Essential Agreement of Gram-positive Clinical and Challenge Isolates Using the Auto Read Method

Clinical Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	112	111	111	110	99.1%	99.1%
Staphylococcus aureus	150	150	150	150	100%	100%
Enterococcus spp.	104	101	102	99	98.1%	98.0%
Streptococcus spp. Beta-hemolytic Group with MHB	78	44	77	43	98.7%	97.7%
Total	**444**	**406**	**440**	**402**	**99.1%**	**99.0%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	17	17	17	17	100.0%	100.0%
Staphylococcus aureus	27	27	27	27	100.0%	100.0%
Enterococcus spp.	16	15	15	14	93.8%	93.3%
Streptococcus spp. Beta-hemolytic Group with MHB	11	8	11	8	100%	100%
Total	**71**	**67**	**70**	**66**	**98.6%**	**98.5%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	129	128	128	127	99.6%	99.6%
Staphylococcus aureus	177	177	177	177	100%	100%
Enterococcus spp.	120	116	117	113	96.0%	95.7%
Streptococcus spp. Beta-hemolytic Group with MHB	89	52	88	51	99.4%	98.9%
Total	**515**	**473**	**510**	**468**	**99.0%**	**98.9%**

Table 6. Summary Data and % Essential Agreement of *Streptococcus* spp. Clinical and Challenge Isolates Using the Manual Read Method

Clinical Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	151	151	150	150	99.3%	99.3%
Streptococcus spp. Viridans Group	104	104	104	104	100.0%	100.0%
Streptococcus spp. Beta-hemolytic Group with LHB	195	194	194	193	99.5%	99.5%
Total	**450**	**449**	**448**	**447**	**99.6%**	**99.6%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	25	25	25	25	100.0%	100.0%
Streptococcus spp. Viridans Group	26	25	26	25	100.0%	100.0%
Streptococcus spp. Beta-hemolytic Group with LHB	25	25	25	25	100.0%	100.0%
Total	**76**	**75**	**76**	**75**	**100.0%**	**100.0%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	176	176	175	175	99.7%	99.7%
Streptococcus spp. Viridans Group	130	129	130	129	100.0%	100.0%
Streptococcus spp. Beta-hemolytic Group with LHB	220	219	219	218	99.5%	99.7%
Total	**526**	**524**	**524**	**522**	**99.6%**	**99.6%**

Table 7. Summary Data and % Essential Agreement of *Streptococcus* spp. Clinical and Challenge Isolates Using the Auto Read Method

Clinical Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	151	151	148	148	98.0%	98.0%
Streptococcus spp. Viridans Group	104	104	104	104	100.0%	100.0%
Streptococcus spp. Beta-hemolytic Group with LHB	195	193	194	192	99.5%	99.5%
Total	**450**	**448**	**446**	**444**	**99.1%**	**99.1%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	25	25	25	25	100.0%	100.0%
Streptococcus spp. Viridans Group	25	25	25	25	100.0%	100.0%
Streptococcus spp. Beta-hemolytic Group with LHB	25	25	25	25	100.0%	100.0%
Total	**75**	**75**	**75**	**75**	**100.0%**	**100.0%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	176	176	173	173	99.0%	99.0%
Streptococcus spp. Viridans Group	129	129	129	129	100.0%	100.0%
Streptococcus spp. Beta-hemolytic Group with LHB	220	218	219	217	99.8%	99.8%
Total	**525**	**523**	**521**	**519**	**99.2%**	**99.2%**

Essential agreement for **telavancin** on the Sensititre® susceptibility plate compared to the CLSI reference microdilution plate was calculated for each method (automated and manual read) using the +/- one $\log_2$ dilution standard. The calculation for Evaluable excluded any test results where MICs were off-scale for the dilutions tested. Essential agreement rates are shown for Gram positive isolates in tables 4 and 5, and for *Streptococcus* spp. isolates in tables 6 and 7.

Clinical Isolates and CDC Challenge Organisms

• **Gram positive Isolates:** The overall essential agreement for **telavancin** (+/- one $\log_2$ dilution) was 99.6% for the manual method and 99.0% for the automated method (Tables 4 and 5).

• ***Streptococcus* spp. Isolates:** The overall essential agreement for **telavancin** (+/- one $\log_2$ dilution) was 99.6% for the manual method and 99.2% for the automated method (Tables 6 and 7).

Interlaboratory Reproducibility

• **Gram positive Isolates:** Reproducibility testing results for **telavancin** (+/- one $\log_2$ dilution) from the modal MIC was 100% for the automated method and 100% for the manual read method (Table 8).

• ***Streptococcus* spp. Isolates:** Reproducibility testing results for **telavancin** (+/- one $\log_2$ dilution) from the modal MIC was 99% for the automated method and 97% for the manual read method (Table 8).

Table 8. Interlaboratory Reproducibility % Essential Agreements +/- one $\log_2$ Dilution of the Modal MIC for Telavancin

	Auto gram-positive	Manual gram-positive	Auto *Streptococcus* spp.	Manual *Streptococcus* spp.
Between-site total isolates tested	75	75	75	75
Between-site isolates within +/- 1 well from mode	75	75	74	73
Between-site reproducibility ratio	75/75	75/75	74/75	73/75
Between-site reproducibility %	100%	100%	99%	97%
Total essential agreement	72	73	73	73
Essential agreement %	96%	97%	97%	97%

Conclusions

This study validates that the Sensititre® 18 – 24 hour susceptibility system (both automated and manual read) demonstrated an equivalent level of performance compared to the CLSI M07 reference broth microdilution plate when testing **telavancin** against Gram-positive and *Streptococcus* spp. clinical and challenge isolates. The high level of essential agreement obtained by the Sensititre® 18 – 24 hour susceptibility method and the CLSI reference method suggests that this is an acceptable method for susceptibility testing of **telavancin**.

References

Clinical and Laboratory Standards Institute. 2009. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-eighth edition.* Approved document M07-A8. Wayne, PA: CLSI.

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