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A Multi-Site Study Comparing an 18-24h Sensititre® Susceptibility System to the CLSI Broth Microdilution Method for Minocycline using Gram-Positive and Gram-Negative Organisms

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Abstract

Background: Minocycline (MIN) is a broad spectrum tetracycline antibiotic, originally developed by Lederle Laboratories, that is used to treat bacterial infections, including pneumonia and other respiratory tract infections. An evaluation was performed to determine the accuracy and reproducibility of MIN 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 methodologies were performed.

Materials and Methods: MIN (0.03-16µg/mL) was tested against 825 clinical isolates. 135 challenge isolates and 50 reproducibility isolates. The isolates consisted of: 177 *Staphylococcus aureus*, 131 *Staphylococcus*. species, 121 *Enterococcus* species, 324 Enterobacteriaciae, 119 non-Enterobacteriaciae, and 88 *Acinetobacter* species. Dried plates were inoculated as per manufacturers' instructions and BMD was performed per CLSI M07. CLSI quality control (QC) organisms were tested daily and all results were within CLSI QC ranges.

Results: Comparisons of MIN MIC results on the Sensititre® system to the CLSI M07 BMD for both automated and manual reads resulted in 98.6% and 99.8% essential agreement (+/- one $\log_2$ dilution), respectively. With categorical agreements, there were no very major or major errors and ≤10% minor error rate for all isolates. The overall agreement for the reproducibility (+/- one $\log_2$ dilution of the modal MIC) for automated and manual reads was 97.5%.

Conclusions: The results for MIN indicates that the Sensititre® 18-24h susceptibility system for all clinical and challenge isolates gave reliable results using either the automated or manual read methods compared to the reference CLSI M07 BMD.

Introduction

Minocycline, a semi-synthetic derivative of tetracycline, is a bacteriostatic antibiotic used to treat strains of methicillin-resistant *Staphylococcus aureus* (MRSA) infection and disease caused by resistant Acinetobacter species. 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 – 24 hour susceptibility system with **minocycline** 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 gram positive and gram negative organisms.

• Each isolate was tested using a Sensititre 18 – 24 hour susceptibility plate containing **minocycline** (0.03-16µg/mL). 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 960 fresh clinical isolates (combined 3 sites); approximately 369 gram positive isolates, and 456 gram negative isolates from each site. 135 Centers for Disease Control and Prevention (CDC) challenge isolates consisted of: 60 gram positive and 75 gram negative supplied to a single testing site (Tables 1 and 2).

• Reproducibility testing consisted of 25 gram positive and 25 gram negative 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 *E.coli* 25922, at each site (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 (combined 3 sites)	
(369 gram-positive, 456 gram-negative)	825
CDC Challenge Isolates (1 site)	
(60 gram-positive, 75 gram-negative)	135
Reproducibility Isolates (combined 3 sites)	
(25 gram-positive, 25 gram-negative)	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
Stahphylococcus spp.	131
Staphylococcus aureus	177
Enterococcus spp.	121
Total	**429**
Gram negative	Number Tested
Enterobacteriaciae	324
Non-Enterobacteriaciae	119
Acintetobacter spp.	88
Total	**531**

Table 3. Quality Control Strains

Quality Control Strains	CLSI MIC Ranges (µg/ml)
Staphylococcus aureus ATCC 29213	0.06-0.5
Enterococcus faecalis ATCC 29212	1-4
Escherichia coli ATCC 25922	0.25-1

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
Staphylococcus spp.	114	112	114	112	100.0%	100.0%
Staphylococcus aureus	150	150	149	149	99.3%	99.3%
Enterococcus spp.	105	103	105	103	100.0%	100.0%
Total	**369**	**365**	**368**	**364**	**99.7%**	**99.7%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Staphylococcus spp.	17	16	17	16	100.0%	100.0%
Staphylococcus aureus	27	27	27	27	100.0%	100.0%
Enterococcus spp.	16	15	16	15	100.0%	100.0%
Total	**60**	**58**	**60**	**58**	**100%**	**100%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Staphylococcus spp.	131	128	131	128	100.0%	100.0%
Staphylococcus aureus	177	177	176	176	99.4%	99.4%
Enterococcus spp.	121	118	121	118	100.0%	100.0%
Total	**429**	**423**	**428**	**422**	**99.8%**	**99.8%**

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
Staphylococcus spp.	112	106	109	103	97.3%	97.2%
Staphylococcus aureus	150	150	149	149	95.2%	96.0%
Enterococcus spp.	104	101	99	97	99.3%	99.3%
Total	**366**	**357**	**357**	**349**	**97.5%**	**97.8%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Staphylococcus spp.	17	16	17	16	100.0%	100.0%
Staphylococcus aureus	27	27	27	27	100.0%	100.0%
Enterococcus spp.	16	15	15	14	93.8%	93.3%
Total	**60**	**58**	**59**	**57**	**98.3%**	**98.3%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Staphylococcus spp.	129	122	126	119	97.7%	97.5%
Staphylococcus aureus	177	177	176	176	99.4%	99.4%
Enterococcus spp.	120	116	114	111	95.0%	95.7%
Total	**426**	**415**	**416**	**406**	**97.6%**	**97.8%**

Table 6. Summary Data and % Essential Agreement of Gram-Negative 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
Enterobacteriaciae	267	225	267	225	100.0%	100.0%
Non-Enterobacteriaciae	107	90	107	90	100.0%	100.0%
Acinetobacter spp.	82	69	81	69	99.8%	100.0%
Total	**456**	**384**	**455**	**384**	**99.8%**	**100.0%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Enterobacteriaciae	57	49	57	49	100.0%	100.0%
Non-Enterobacteriaciae	12	4	12	4	100.0%	100.0%
Acinetobacter spp.	6	5	6	5	100.0%	100.0%
Total	**75**	**58**	**75**	**58**	**100.0%**	**100.0%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Enterobacteriaciae	324	274	324	274	100.0%	100.0%
Non-Enterobacteriaciae	119	94	119	94	100.0%	100.0%
Acinetobacter spp.	88	74	87	74	98.9%	100.0%
Total	**531**	**442**	**530**	**442**	**99.8%**	**100.0%**

Table 7. Summary Data and % Essential Agreement of Gram-Negative 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
Enterobacteriaciae	266	223	265	222	99.6%	99.6%
Non-Enterobacteriaciae	107	84	107	84	100.0%	100.0%
Acinetobacter spp.	82	70	80	69	97.6%	98.6%
Total	**455**	**377**	**452**	**375**	**99.3%**	**99.5%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Enterobacteriaciae	57	49	57	49	100.0%	100.0%
Non-Enterobacteriaciae	12	4	12	4	100.0%	100.0%
Acinetobacter spp.	6	5	6	5	100.0%	100.0%
Total	**75**	**58**	**75**	**58**	**100.0%**	**100.0%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Enterobacteriaciae	323	272	322	271	99.7%	99.6%
Non-Enterobacteriaciae	119	88	119	88	100.0%	100.0%
Acinetobacter spp.	88	75	86	74	97.8%	98.7%
Total	**530**	**435**	**527**	**433**	**99.4%**	**99.5%**

Essential agreement for **minocycline** on the Sensititre® susceptibility plate compared to the CLSI reference microdilution plate was calculated for each method (manual and autoread read) using the +/- one $\log_2$ dilution standard. The calculations 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 gram negative isolates in tables 6 and 7.

Clinical Isolates and CDC Challenge Organisms

• **Gram positive Isolates:** The overall essential agreement for **minocycline,** within +/- one $\log_2$ dilution, was 99.8% for the manual method and 97.6% for the autoread method (Tables 4 and 5).

• **Gram negative Isolates:** The overall essential agreement for **minocycline,** within +/- one $\log_2$ dilution, was 99.8% for the manual method and 99.4% for the autoread method (Tables 6 and 7).

Interlaboratory Reproducibility

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

• **Gram negative Isolates:** Reproducibility testing results for **minocycline**, within +/- one $\log_2$ dilution from the modal MIC was, 99% for the autoread 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 Minocycline

	Auto gram-positive	Manual gram-positive	Auto gram-negative	Manual gram-negative
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 manual and autoread read) demonstrated an equivalent level of performance compared to the CLSI M07 reference broth microdilution plate when testing **minocycline** against gram positive and gram negative 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 **minocycline**.

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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