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A Multi-Site Study Comparing a Commercially Prepared Dried MIC Susceptibility System to the CLSI Broth Microdilution Method for Delafloxacin using non-Fastidious Gram-Positive Organisms

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ABSTRACT

Background: Delafloxacin (DLX) (Melinta Therapeutics, New Haven, CT), is a fluoroquinolone antibiotic that has completed clinical development and is under review by the FDA for acute bacterial skin and skin structure infections (ABSSSI) indication. It is more active than other quinolones against non-fastidious Gram-positive pathogens including *Staphylococcus aureus* (MRSA and MSSA), Coagulase-negative *Staphylococcus* spp. (CoNS), and *Enterococcus faecalis* (VRE and VSE). A 4 site evaluation was designed to determine the accuracy and reproducibility of DLX susceptibility testing using the Sensititre® dried MIC susceptibility system (Thermo Fisher Scientific, Cleveland, OH) compared with the CLSI (M07)/ ISO 20776-1 reference broth microdilution method (BMD). Both auto and manual read methodologies were employed.
Materials and Methods: DLX (0.0005-8 µg/ml) was tested against 508 recent clinical isolates, 112 challenge isolates and 56 reproducibility isolates. These isolates consisted of *S. aureus* (272 MRSA and 268 MSSA), 63 CoNS, and 73 *Enterococcus faecalis.* The Sensititre dried MIC susceptibility system was inoculated per manufacturers' instructions. BMD was performed per CLSI (M07)/ISO guidelines. Recommended CLSI quality control (QC) organisms were tested daily and all results were within the published QC ranges.
Results: Comparisons of DLX MIC values of non-fastidious Gram-positive MIC results on the Sensititre system to the CLSI/ISO BMD for automated and manual reads resulted in 97.3% and 99.8% essential agreement (EA; +/- 1 $\log_2$ dilution) respectively. Overall agreement for the reproducibility (+/- 1 $\log_2$ dilution of the modal MIC) using automated and manual reads was 99.1% and 99.6%, respectively.
Conclusions: The results of this DLX study on the Sensititre susceptibility system (both auto and manual read) demonstrated an equivalent level of performance compared to the CLSI /ISO BMD when testing DLX against *S. aureus*, CoNS, and *Enterococcus faecalis*. The high level agreement obtained by the Sensititre susceptibility system and the BMD suggests that this is an acceptable method for susceptibility testing of DLX.

INTRODUCTION and OBJECTIVES

Delafloxacin (DLX) (Figure1.) a novel fluoroquinolone, developed by Melinta Therapeutics, was recently submitted to the FDA for the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by isolates of the following Gram-positive microorganisms: *Staphylococcus aureus* (including methicillin-resistant [MRSA] and methicillin-susceptible [MSSA] isolates), Coagulase-negative *Staphylococcus* spp. and *Enterococcus faecalis*. This *in vitro* multi-site comparison study was done to evaluate the performance of Delafloxacin on the commercially manufactured Sensititre® 18-24 hour susceptibility system, for both automated and manual read, compared against the Clinical Laboratory Standards Institute (CLSI) reference broth microdilution (BMD) method (M07) and ISO 20776-1. As series of studies were conducted to establish equivalency between the two methods. MIC results obtained using the Sensititre dried plate technology were compared to the MIC results obtained from the CLSI frozen reference plate.

MATERIALS and METHODS

- The Sensititre 18-24 hour MIC susceptibility system (Thermo Fisher Scientific, Oakwood Village, OH) is an *in vitro* diagnostic product for clinical susceptibility testing of both fastidious and non-fastidious organisms.

Delafloxacin was tested against:

- 508 recent clinical isolates across the four sites
- 112 challenge isolates at a single testing site
- 14 reproducibility isolates at each site (tested in triplicate over a 3 day testing period)
- 2 Quality Control Strains (ATCC)

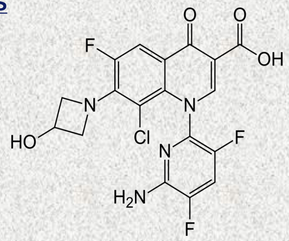


Figure 1. Chemical Structure of Delafloxacin

MATERIALS and METHODS Cont.

- Colony Counts were performed on the inoculums of the Clinical, Challenge, Reproducibility and QC strains on each day of testing.

- Each isolate was tested using a:
  - Dried Sensititre® 18–24 susceptibility plate containing Delafloxacin (0.0005-8µg/ml). The dried plates were set up and tested by both automated and manual reading methodologies according to the manufacturer's instructions.

  - CLSI reference broth microdilution plate was prepared and tested on each isolate according to the current Clinical Laboratory Standards Institute standard method (CLSI M07/M100 and ISO 20776-1).

Table 1. Organisms Tested	Number Tested
Clinical Isolates (4 sites)	508
CDC Challenge Isolates (one site)	112
Reproducibility Isolates (4 sites) (3 x day for 3 days)	14 (504)
ATCC Quality Control Strains (20 replicates of each strain at 4 sites)	2 (160)
TOTAL	**1284**

Quality Control

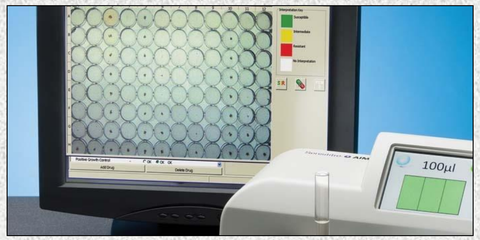
- Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges.

- Colony counts were performed and fell within expected ranges Reference 2-8X10^5, Sensititre 5X10^4-5X10^5

Table 2. Quality Control Strains	CLSI DLX QC Ranges (µg/ml)
Staphylococcus aureus ATCC 29213	0.001-0.008
Enterococcus faecalis ATCC 29212	0.015-0.12

RESULTS

Essential agreement for **Delafloxacin** on the Sensititre susceptibility plate compared to the CLSI reference microdilution plate was calculated for each read method (Auto and Manual) using the +/- one $\log_2$ dilution standard. Essential agreement rates are shown for non-fastidious gram positive isolates in **Tables 3 and 4**.



RESULTS Cont.

Clinical Isolates and Challenge Organisms

Non-Fastidious Gram-positive Isolates:
The overall essential agreement for Delafloxacin within ±1 $\log_2$ dilution was **99.8 %** for the manual method and **97.3%** for the auto read method.

Interlaboratory Reproducibility

Non-Fastidious Gram-positive Isolates:
Reproducibility testing results for Delafloxacin within ±1 $\log_2$ dilution from the modal MIC was **99.1%** for the auto read method and **99.6%** for the manual read method.

Table 3. Summary Data and % Essential Agreement of Non-Fastidious Gram-positive Clinical and Challenge Isolates Using the Manual Read Method

The overall essential agreement for Delafloxacin within +/- one $\log_2$ dilution, was 99.8% for the manual read method

Combined Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	[1]Evaluable	Total	[1]Evaluable	Total	[1]Evaluable
Staphylococcus aureus (MRSA)	252	252	252	252	100%	100%
Staphylococus aureus (MSSA)	248	244	247	244	99.6%	100%
Enterococcus spp.	65	65	65	65	100%	100%
Coagulase-negative *Staphylococcus* spp.	55	55	55	55	100%	100%
Total	**620**	**616**	**619**	**616**	**99.8%**	**100%**

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

The overall essential agreement for Delafloxacin within +/- one $\log_2$ dilution, was 97.3% for the auto read method

Combined Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	[1]Evaluable	Total	[1]Evaluable	Total	[1]Evaluable
Staphylococcus aureus (MRSA)	252	252	248	248	98.4%	98.4%
Staphylococus aureus (MSSA)	248	244	237	236	95.6%	96.7%
Enterococcus spp.	65	65	64	64	98.5%	98.5%
Coagulase-negative *Staphylococcus* spp.	55	55	54	54	98.2%	98.2%
Total	**620**	**616**	**603**	**602**	**97.3%**	**97.7%**

Footnotes
[1]When the reference method result is on-scale and the new device result is also on scale. FDA believes that if the reference result is on-scale, and the new device result is not on scale, comparative data may not be evaluable. FDA does not consider evaluable any reference result that falls in the less than or greater than category. However, such results may be a part of the EA and/or CA assessment.

RESULTS Cont.

Table 5. Interlaboratory Reproducibility % Essential Agreement ±1 $\log_2$ dilution from the Modal Value

Delafloxacin	Auto Read	Manual Read
Between-site total isolates tested	504	504
Between-site isolates within +/- 1 well from mode	498	502
Between-site reproducibility ratio	498	502
Between-site reproducibility %	**99.1%**	**99.6%**
Total essential agreement	498/504	502/504
Essential agreement %	**99.1%**	**99.6%**

CONCLUSIONS

This study validates that the Sensititre 18–24 hour susceptibility system (both auto read and manual read) demonstrated an equivalent level of performance compared to the reference broth microdilution plate when testing **Delafloxacin** against non-fastidious Gram positive clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of **Delafloxacin**.

REFERENCES

Clinical and Laboratory Standards Institute. 2015. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-tenth edition.* Approved document M07-A10. Wayne, PA: CLSI.

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"The NDA (New Drug Application) for Delafloxacin is under FDA review"