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A Multi-Site Study Comparing a Commercially Prepared Dried MIC Susceptibility System to the CLSI/ISO Broth Microdilution Method for Eravacycline using Gram Positive and Gram Negative Non-Fastidious Organisms

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ABSTRACT

Background: Eravacycline (ERV) (Tetraphase Pharmaceuticals, Watertown, MA) is a novel, fully-synthetic fluorocycline displaying broad spectrum activity against non-fastidious organisms, including multidrug-resistant bacteria. A 4-site study was performed to determine the accuracy and reproducibility of ERV susceptibility testing against gram positive and gram negative non-fastidious organisms using the Thermo Scientific Sensititre™ dried MIC susceptibility system (Thermo Fisher Scientific, Cleveland, OH) compared with the CLSI (M07/M100) and ISO 20776-1, (CLSI/ISO) reference broth microdilution method (BMD). Both automated and manual reading methodologies were tested.
Methods: ERV (0.001-16µg/mL) was tested against 848 recent clinical isolates, 180 challenge isolates, and 28 reproducibility isolates consisting of: *Staphylococcus aureus* (MRSA, 254), *Staphylococcus aureus* (MSSA, 256), *Enterococcus* spp. (132), *E. coli* (122), *Klebsiella* spp. (166), *Enterobacter* spp. (77), *Citrobacter* spp. (49). The Sensititre dried MIC susceptibility system was inoculated per manufacturers' instructions and the BMD method was performed per CLSI (M07/M100) and ISO 20776-1 guidelines. CLSI quality control organisms were tested daily and were within the published ranges.
Results: Comparison of gram positive non-fastidious MIC results on the Sensititre system to the CLSI/ISO BMD method for automated and manual reads resulted in 99.2% and 98.9% essential agreement (EA, +/- 1 log_2 dilution) for ERV, respectively. Overall the essential agreements for reproducibility (+/- 1 log_2 dilution of the modal MIC) using automated and manual reads were 100% and 100%. Comparison of gram negative non-fastidious MIC results on the Sensititre system to the CLSI/ISO BMD method for automated and manual reads resulted in 100% and 100% essential agreement (EA, +/- 1 log_2 dilution) for ERV, respectively. Overall the essential agreements for reproducibility (+/- 1 log_2 dilution of the modal MIC) using automated and manual reads were 99.8% and 99.4%.
Conclusion: The Sensititre susceptibility system demonstrates an equivalent level of performance compared to the CLSI/ISO BMD method when testing ERV against gram positive and gram negative non-fastidious organisms. The high level of agreement obtained by the Sensititre susceptibility system and the CLSI/ISO BMD method suggests that this is an acceptable method for susceptibility testing of ERV.

INTRODUCTION

Eravacycline is a novel, fully-synthetic fluorocycline antibiotic with a broad spectrum of activity against a variety of gram-positive and gram-negative bacteria including multi drug resistant strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant Enterobacteriaceae spp. This *in vitro* multi-site comparison study was done to evaluate the performance of eravacycline on the commercially manufactured Sensititre 18-24 hour susceptibility system, for both automated and manual reads, compared against the Clinical Laboratory Standards Institute (CLSI) reference broth microdilution (BMD) method (M07/M100) and ISO 20776-1 (BMD). To establish equivalency between the two methods, a four lab clinical study was conducted, and the MIC results obtained using the Sensititre dried plate technology were compared to the MIC results obtained from the CLSI M07/M100 frozen reference plate.

MATERIALS AND METHODS

- The Sensititre 18-24 hour 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.

Eravacycline was tested against: (Table 1.)

- 848 recent clinical isolates across the four sites
- 180 challenge isolates at a single testing site
- 28 reproducibility isolates at each site (tested in triplicate over a 3 day testing period)
- 4 Quality Control Strains (ATCC)

MATERIALS AND METHODS Cont.

- Colony Counts and purity plates 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 non-fastidious gram negative plate containing eravacycline at 0.008-16 µg/ml and a non-fastidious gram positive plate containing eravacycline at 0.001-16 µg/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.

Table 1. Organisms Tested	Number Tested
Clinical Isolates (4 sites)	848
CDC Challenge Isolates (one site)	180
Reproducibility Isolates (4 sites) (3 x day for 3 days)	28 (1008)
ATCC Quality Control Strains (20 replicates of each strain at 4 sites)	4 (320)
TOTAL	**2356**

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 QC Ranges (µg/ml)
Staphylococcus aureus ATCC 29213	0.015-0.12
Enterococcus faecalis ATCC 29212	0.015-0.06
Escherichia coli ATCC 25922	0.03-0.12
Pseudomonas aeruginosa ATCC 27853	2-16

Results

Essential agreement for **eravacycline** on the Sensititre susceptibility plate compared to the 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-negative isolates in **Table 3**. Essential agreement rates are shown for gram positive non-fastidious isolates in **Table 4**.

RESULTS Cont.

Clinical Isolates and Challenge Organisms

Gram Positive non-Fastidious
The overall essential agreement for eravacycline within ±1 log_2 dilution was **98.9%** for the manual method and **99.2%** for the auto read method.
Gram Negative non-Fastidious
The overall essential agreement for eravacycline within ±1 log_2 dilution was **100%** for the manual method and **100%** for the auto read method.

Reproducibility Organisms

Gram Positive non-Fastidious Inter-laboratory Reproducibility
Reproducibility testing results for eravacycline within ±1 log_2 dilution from the modal MIC was **100%** for the auto read method and **100%** for the manual read method
Gram Negative non-Fastidious Inter-laboratory Reproducibility
Reproducibility testing results for eravacycline within ±1 log_2 dilution from the modal MIC was **99.8%** for the auto read method and **99.4%** for the manual read method.

Table 3. Summary Data and % Essential Agreement of Gram Negative non-Fastidious Clinical and Challenge Isolates Using the Auto and Manual Read Methods

Combined Total Isolates

Eravacycline	% Essential Agreement	
Organism Group	**Auto Read Method**	**Manual Read Method**
Escherichia coli	100%	100%
Klebsiella pneumoniae	100%	100%
Klebsiella oxytoca	100%	100%
Enterobacter spp.	100%	100%
Citrobacter spp.	100%	100%
Stenotrophomonas maltophilia	100%	100%
Acinetobacter baumannii	100%	100%
Total	**100%**	**100%**

Table 4. Summary Data and % Essential Agreement of Gram Positive non-Fastidious Clinical and Challenge Isolates Using the Auto and Manual Read Methods

Combined Total Isolates

Eravacycline	% Essential Agreement	
Organism Group	**Auto Read Method**	**Manual Read Method**
Staphylococcus aureus (MRSA)	99.2%	98.8%
Staphylococcus aureus (MSSA)	100%	100%
Enterococcus spp.	97.6%	96.8%
Total	**99.2%**	**98.9%**

RESULTS Cont.

Table 5a. Gram Negative non-Fastidious Inter-laboratory Reproducibility % Essential Agreement ±1 log_2 dilution from the Modal Value

Eravacycline	Auto Read	Manual Read
Between-site total isolates tested	540	540
Between-site isolates within +/- 1 well from mode	539	537
Between-site reproducibility ratio	539	537
Between-site reproducibility %	**99.8%**	**99.4%**
Total essential agreement	539/540	537/540
Essential agreement %	**99.8%**	**99.4%**

Table 5b. Gram Positive non-Fastidious Inter-laboratory Reproducibility % Essential Agreement ±1 log_2 dilution from the Modal Value

Eravacycline	Auto Read	Manual Read
Between-site total isolates tested	468	468
Between-site isolates within +/- 1 well from mode	468	468
Between-site reproducibility ratio	468	468
Between-site reproducibility %	**100%**	**100%**
Total essential agreement	468/468	468/468
Essential agreement %	**100%**	**100%**

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 CLSI M07/M100 reference broth microdilution plate when testing **eravacycline** against non-fastidious gram negative and gram positive clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of **eravacycline**.

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