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A Multi-Site Study Comparing a Commercially Prepared Dried MIC Susceptibility System to the CLSI Broth Microdilution Method for Eravacycline using 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 a variety of organisms. A 4-site study was performed to determine the accuracy and reproducibility of ERV susceptibility testing against *Streptococcus* spp. and *Haemophilus influenzae* using the Thermo Scientific™ Sensititre® dried MIC susceptibility system (Thermo Fisher Scientific, Cleveland, OH) compared with the CLSI (M07) reference broth microdilution method (BMD).
Methods: ERV (0.002-16µg/mL) was tested against 537 recent clinical isolates, 118 challenge isolates, and 15 reproducibility isolates of *Streptococcus* spp. These isolates consisted of *S. pneumoniae* (253), *S. pyogenes* (121), *S. agalactiae* (130), *S. mitis* group (62), *S. salivarius* (35), *S. anginosus* group (69). ERV (0.001-16µg/mL) was also tested against 393 recent clinical isolates, 50 challenge isolates, and 10 reproducibility isolates of *H. influenzae* (beta lactamase positive and negative). The Sensititre MIC susceptibility system was inoculated per manufacturers' instructions, and the BMD method was performed per CLSI guidelines. Quality control organisms were tested daily and were within acceptable ranges.
Results: Comparison of *Streptococcus* spp. MIC results on the Sensititre system to the CLSI BMD method for automated and manual reads resulted in 99.4% and 99.2% 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 98.5% and 98.9%. Comparison of *H. influenzae* MIC results on the Sensititre system to the CLSI BMD method for manual read methodology resulted in 98.6% essential agreement (EA, +/- 1 log_2 dilution) for ERV. Overall agreements for reproducibility (+/- 1 log_2 dilution of the modal MIC) using manual read methodology was 100%.
Conclusion: The Sensititre susceptibility system demonstrates an equivalent level of performance compared to the CLSI BMD method when testing ERV against fastidious organisms, specifically *Streptococcus* spp., and *H. influenzae*. The high level of agreement obtained by the Sensititre susceptibility system and the CLSI BMD method suggests that this is an acceptable method for susceptibility testing of ERV.

Introduction

Eravacycline (Figure 1.) (Tetraphase Pharmaceuticals, Watertown, MA) is a novel, fully-synthetic fluorocycline displaying broad spectrum activity against a variety of organisms. This *in vitro* multi-site comparison study was conducted to evaluate the performance of *Streptococcus* spp. and *Haemophilus influenzae* (beta lactamase positive and negative) with eravacycline on the commercially manufactured Sensititre® 18-24 hour susceptibility system compared against the Clinical Laboratory Standards Institute (CLSI) reference broth microdilution method (BMD) (M07/M100). To establish equivalency, 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 frozen reference plate.

Materials and methods

●The Sensititre 18-24 hour MIC or breakpoint 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.)

- 537 recent *Streptococcus* spp. and 393 *Haemophilus influenzae* clinical isolates across the four sites
- 118 *Streptococcus* spp. and 50 *Haemophilus influenzae* challenge isolates at a single testing site
- 15 *Streptococcus* spp. and 10 *Haemophilus influenzae* reproducibility isolates at each site (tested in triplicate over a 3 day testing period)
- 2 Quality Control Strains (ATCC)

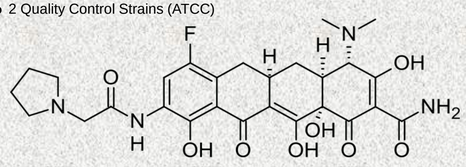

Figure 1. Chemical Structure of Eravacycline

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 plate containing eravacycline (0.002-16µg/ml) for *Streptococcus* spp. and (0.001-16µg/ml) for *Haemophilus influenzae*. The dried plates for *Streptococcus* spp. were set up and tested according to the manufacturer's instructions. Both automated and manual reading methodologies for *Streptococcus* spp. were employed. The dried plates for *Haemophilus influenzae* were set up and tested according to the manufacturer's instructions and was manual read only.

● 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) *Streptococcus* spp.	537
Haemophilus influenzae	393
CDC Challenge Isolates (one site) *Streptococcus* spp.	118
Haemophilus influenzae	50
Reproducibility Isolates (4 sites) (3 x day for 3 days) *Streptococcus* spp.	15 (540)
Haemophilus influenzae	10 (360)
ATCC Quality Control Strains (20+ replicates of each strain at 4 sites) *Streptococcus* spp.	1 (80)
Haemophilus influenzae	1(80)
TOTAL	**2158 (tests)**

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)
Streptococcus pneumoniae ATCC 49619	0.004-0.03µg/ml
Haemophilus influenzae ATCC 49247	0.06-0.5µg/ml

Results

Clinical Isolates and Challenge Organisms

The overall essential agreement for *Streptococcus* spp. within ±1 log_2 dilution was **99.2%** for the manual method and **99.4%** for the auto read method.

The overall essential agreement for *Haemophilus influenzae* within ±1 log_2 dilution was **98.6%** for the manual method.

Inter-laboratory Reproducibility

Reproducibility testing results for *Streptococcus* spp. within ±1 log_2 dilution from the modal MIC was **98.5%** for the auto read method and **98.9%** for the manual read method. **(Table 5.)**

Reproducibility testing results for *Haemophilus influenzae* within ±1 log_2 dilution from the modal MIC was **100.0%** for the manual read method. **(Table 6.)**

Essential agreement for **eravacycline** on the Sensititre susceptibility plate compared to the reference microdilution plate was calculated for each read method using the +/- one log_2 dilution standard. Essential agreement rates for *Streptococcus* spp. are shown in **Table 3.** Essential agreement rates for *Haemophilus influenzae* are shown in **Table 4.** (manual read only).

Table 3. Summary Data and % Essential Agreement of *Streptococcus* spp. Clinical and Challenge Isolates Using the Auto and Manual Read Method

Clinical and Challenge Isolates Combined

Eravacycline	Number of Isolates	Essential Agreement %	
Organism Group	**Total**	**Auto Read**	**Manual Read**
Streptococcus pyogenes	120	98.3%	99.2%
Streptococcus agalactiae	126	100%	100%
Streptococcus anginosus Group	67	100%	97.0%
Streptococcus mitis	58 (*57)	98.2%	98.3%
Streptococcus salivarius	34	100%	100%
Streptococcus pneumoniae	250	99.6%	99.6%
Total	**655**	**99.4%**	**99.2%**

*No MIC for *Streptococcus mitis* isolate MC447 (Optiread did not signal)

Table 4. Summary Data and % Essential Agreement of *Haemophilus influenzae* Clinical and Challenge Isolates Using the Manual Read Method

Clinical and Challenge Isolates Combined

Eravacycline	Number of Isolates	Essential Agreement %
Organism Group	**Total**	**Manual Read**
Haemophilus influenzae	443	437
Total	**443**	**98.6%**

Results Cont.

Table 5. Inter-laboratory Reproducibility for *Streptococcus* spp. % 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	532	533
Between-site reproducibility ratio	532	533
Between-site reproducibility %	**98.5%**	**98.9%**
Total essential agreement	532/540	533/540
Essential agreement %	**98.5%**	**98.9%**

Table 6. Inter-laboratory Reproducibility for *Haemophilus influenzae*. % Essential Agreement ±1 log_2 dilution from the Modal Value

Eravacycline	Manual Read
Between-site total isolates tested	360
Between-site isolates within +/- 1 well from mode	360
Between-site reproducibility ratio	360
Between-site reproducibility %	**100%**
Total essential agreement	360/360
Essential agreement %	**100%**

Conclusions

This study validates that the Sensititre 18–24 hour susceptibility system demonstrated an equivalent level of performance compared to the CLSI M07/M100 reference broth microdilution plate when testing **eravacycline** against *Streptococcus* spp. and *Haemophilus influenzae* clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of **eravacycline**.

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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