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A multi-site study comparing a commercially prepared dried MIC susceptibility system to the CLSI broth microdilution method for omadacycline using non-fastidious Gram-positive organisms

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ABSTRACT

Background: Omadacycline (OMC) (Paratek Pharmaceuticals, King of Prussia, PA), is an antibiotic belonging to the aminomethlycycline subclass of tetracyclines and is in phase 3 development for the treatment of acute bacterial skin and skin structure infections (ABSSSI) and community-acquired bacterial pneumonia (CABP). OMC is active against non-fastidious Gram-positive pathogens including *Staphylococcus aureus* (MRSA and MSSA) and *Enterococcus* species (VRE and VSE). A 4 site evaluation was performed to determine the accuracy and reproducibility of OMC susceptibility testing using the Thermo Scientific™ 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: OMC (0.03-32 µg/mL) was tested against 603 recent clinical isolates, 125 challenge isolates and 15 reproducibility isolates. These isolates consisted of *S. aureus* (254 MRSA and 256 MSSA), 101 Coagulase-negative *Staphylococcus* spp. (CNS), and 132 *Enterococcus spp.* (VSE and VRE). The Sensititre dried MIC susceptibility panels were inoculated per manufacturer's instructions. BMD was performed per CLSI (M07) guidelines. Recommended CLSI quality control (QC) organisms were tested daily and all results were within the published QC ranges.
Results: Comparisons of OMC MICs for non-fastidious Gram-positive MIC results on the Sensititre system to the CLSI BMD for automated and manual reads resulted in 97.7% and 97.8% essential agreements (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 99.8% and 99.4%, respectively.
Conclusions: The results of this OMC study on the Sensititre susceptibility system (both auto and manual read) demonstrated an equivalent level of performance compared to the CLSI BMD when tested against *S. aureus*, CNS, and *Enterococcus* spp. This high level of agreement obtained for both clinical and challenge isolates indicate that reliable results are obtained for OMC on the Sensititre system.

INTRODUCTION AND OBJECTIVES

Omadacycline (Figure 1.) is an aminomethylcycline antibiotic developed by Paratek Pharmaceuticals in development for the treatment of acute bacterial skin and skin structure infections (ABSSSI), community-acquired bacterial pneumonia (CABP), and urinary tract infections (UTI). This *in vitro* multi-site comparison study was done to evaluate the performance of Omadacycline 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 (BMD). To establish equivalency between the two methods, a series of studies were conducted, and the MIC results obtained using the Sensititre dried plate technology were compared to the MIC results obtained from the CLSI frozen reference plate.

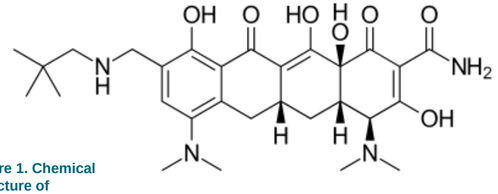

Figure 1. Chemical Structure of Omadacycline

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.
- Omadacycline was tested against: (Table 1.)
  - 603 recent clinical isolates across the four sites
  - 15 reproducibility isolates at each site (tested in triplicate over a 3 day testing period)
  - 2 Quality Control Strains (ATCC)
- 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 Omadacycline (0.03-32µg/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)	603
CDC Challenge Isolates (one site)	125
Reproducibility Isolates (4 sites) (3 x day for 3 days)	15 (540)
ATCC Quality Control Strains (20 replicates of each strain at 4 sites)	2 (160)
TOTAL	**1428**

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 OMC QC Ranges (µg/ml)
Staphylococcus aureus ATCC 29213	0.12-1
Enterococcus faecalis ATCC 29212	0.06-0.5

RESULTS

Essential agreement for **Omadacycline** 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-positive isolates in **Tables 3 and 4**.

CLINICAL ISOLATES AND CHALLENGE ORGANISMS

The overall essential agreement for Omadacycline within ±1 $\log_2$ dilution was **97.8 %** for the manual method and **97.7%** for the auto read method.

INTERLABORATORY REPRODUCIBILITY

Reproducibility testing results for Omadacycline 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 non-fastidious Gram-positive clinical and challenge isolates using 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)	249	249	249	249	100%	100%
Staphylococcus aureus (MSSA)	251	251	248	248	98.8%	98.8%
Enterococcus spp.	129	120	120	118	93.0%	98.3%
Coagulase-negative *Staphylococcus* spp.	99	99	95	95	96.0%	96.0%
Total	**728**	**719**	**712**	**710**	**97.8%**	**98.7%**

The overall essential agreement for Omadacycline within +/- one log2 dilution, was 97.8% for the manual read method

Table 4. Summary data and % essential agreement of non-fastidious Gram-positive clinical and challenge isolates using 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)	249	249	247	247	99.2%	99.2%
Staphylococcus aureus (MSSA)	251	251	245	245	97.6%	97.6%
Enterococcus spp.	129	121	123	120	95.3%	99.2%
Coagulase-negative *Staphylococcus* spp.	98	98	95	95	96.9%	96.9%
Total	**727**	**719**	**710**	**707**	**97.7%**	**98.3%**

Challenge isolate GPCH0113 *Staphylococcus saprophyticus* did not generate a signal on the Optiread

The overall essential agreement for Omadacycline within +/- one $\log_2$ dilution, was 97.7% for the auto read method.

Table 5. Interlaboratory reproducibility % essential agreement ±1 $\log_2$ dilution from the modal value

Omadacycline	Auto Read	Manual Read
Between-site total isolates tested	440	440
Between-site isolates within +/- 1 well from mode	439	437
Between-site reproducibility ratio	439	437
Between-site reproducibility %	**99.8%**	**99.4%**
Total essential agreement	439/440	437/440
Essential agreement %	**99.8%**	**99.4%**

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 Omadacycline against non-fastidious Gram-positive clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of Omadacycline.

REFERENCES

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[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.