Presentation No. 2854 JUNE 12, 2022

An Equivalency Study for Omadacycline Comparing a Commercially Prepared Dried MIC Susceptibility System to the CLSI Broth Microdilution Method Using Rapidly Growing Non-Tuberculous Mycobacteria

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Abstract

Background: Omadacycline (OMC) (Paratek Pharmaceuticals, Inc., King of Prussia, PA) is a novel aminomethylcycline, tetracycline class antibiotic, that binds the 30S ribosomal subunit to block protein synthesis. OMC has been FDA approved for use in treatment of community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSSI) caused by Gram-positive and Gram-negative aerobic and atypical bacteria. Omadacycline is currently being investigated for the treatment of infections caused by rapidly growing non-tuberculous mycobacteria (NTM). An evaluation was performed to determine the accuracy and reproducibility of OMC susceptibility testing using the Sensititre™ dried MIC susceptibility system (Thermo Fisher Scientific™, East Grinstead, UK) compared with the CLSI M24 reference broth microdilution method (BMD) (Thermo Fisher Scientific, Cleveland, OH).

Materials and Methods: 205 recent clinical isolates were tested against OMC (0.008-8µg/mL) and two comparators, tigecycline (0.008-8µg/mL) and doxycycline (0.06-16µg/mL).These isolates consisted of 50 M. *abscessus subsp. abscessus,* 30 *M. abscessus subsp. massiliense,* 5 *M. abscessus subsp. bolletii,* 35 *M. chelonae,* 35 *M. fortuitum,* 30 *M. mucogenicum,* and 20 *M. immunogenum* isolates. A subset of 12 isolates were tested for reproducibility. The Sensititre dried MIC susceptibility panels were inoculated per manufacturer's instructions. Reference BMD plates were tested per CLSI guidelines. Recommended CLSI quality control (QC) organisms were tested concurrently with clinical isolates.

Results: Comparisons of OMC MICs for rapidly growing NTM on the Sensititre system to the CLSI BMD MICs resulted in a 99.5% essential agreement (EA; +/- 1 $\log_2$ dilution). Essential agreements of the comparators, tigecycline and doxycycline, were 98% and 98.5% respectively. Overall agreement for reproducibility (+/- 1 $\log_2$ dilution of the modal MIC) was 100% for OMC, 95.4% for tigecycline, and 99.1% for doxycycline.

Conclusions: The Sensititre susceptibility system demonstrated an equivalent level of performance compared to the CLSI BMD reference method when tested against rapidly growing NTM strains. This high level of agreement indicates that comparable results were obtained for Omadacycline on the Sensititre system, showing that the Sensititre system is an acceptable platform for the testing of Omadacycline against rapidly growing non-tuberculous mycobacterial isolates.

Introduction

OMC is a first-in-class aminomethylcycline with the ability to overcome many of the resistance mechanisms other tetracycline class antibiotics are vulnerable to. OMC has been FDA approved for use against Gram-positive and Gram-negative bacteria that cause community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSSI) and is currently being investigated for treatment of infections caused by rapidly growing non-tuberculous mycobacteria (NTM).

An in-house validation study was done to evaluate the performance of OMC on the commercially prepared dried Sensititre susceptibility plate compared to the CLSI broth microdilution (BMD) frozen reference method (CLSI M24). The evaluation consisted of 205 rapidly growing non-tuberculous mycobacterial clinical isolates. Additionally, a selection of twelve isolates were tested on the dried plates in triplicate over three days to assess the reproducibility of results on the Sensititre system (**Table 4**). Three CLSI recommended quality control organisms were run concurrently (**Table 2**).

Materials and methods

- Sensititre dried MIC susceptibility plates (Thermo Fisher Scientific, East Grinstead, UK) (**Fig. 1**)
- CLSI M24 reference broth microdilution method (BMD) plates (Thermo Fisher Scientific, Cleveland, OH)
- 205 rapidly growing NTM clinical isolates (**Table 1**)
- 3 quality control strains (**Table 2**)
- 12 reproducibility isolates (**Table 4**)

Materials and methods (cont.)

Table 1. Organisms Tested

Species	Quantity
M. abscessus subsp. abscessus (M. abs. abs)	50
M. abscessus subsp. bolletii (M. abs. bol.)	5
M. abscessus subsp. massiliense (M. abs. mass.)	30
M. chelonae	35
M. fortuitum	35
M. immunogenum	20
M. mucogenicum	30
Total	205

All 205 clinical isolates were tested against OMC (0.008-8µg/mL) and two comparators, tigecycline (TGC) (0.008-8µg/mL) and doxycycline (DOX) (0.06-16µg/mL), on both the Sensititre dried plates and the frozen reference BMD plates. Dried plates were tested according to manufacturer's instructions. Frozen reference plates were tested according to CLSI M24. Plates were read manually using the Sensititre™ Manual Viewbox (**Fig. 2**).

Figure 1. Sensititre Dried MIC Susceptibility Plates

Figure 2. Sensititre Manual Viewbox

Table 2. Quality Control Strains

Quality Control Strain	CLSI QC Ranges (µg/ml)		
	OMC	TGC	DOX
Mycobacterium peregrinum ATCC 700686	0.06-0.25	0.03-0.25	0.12-0.5
Staphylococcus aureus ATCC 29213	0.12-1	0.03-0.25	0.12-0.5
Enterococcus faecalis ATCC 29212	0.06-0.5	0.03-0.12	2-8

Recommended CLSI quality control organisms were tested daily and were within the expected ranges. Colony counts were performed daily for each QC strain to verify the isolates correct inoculum concentration.

Results

Essential agreement

Essential agreement for OMC on the Sensititre susceptibility plate compared to the frozen reference microdilution plate was calculated using the ±1 $\log_2$ dilution standard. Isolates that had more than one well variation were re-tested in triplicate to establish a true MIC.

The essential agreement of OMC after repeat testing was 99.5% (**Table 3**). Essential agreements of TGC and DOX were 98% and 98.5% respectively.

Results (cont.)

Table 3. Summary Data and % Essential Agreement of Rapidly Growing Non-tuberculous Mycobacteria Clinical Isolates After Repeat Testing

Organism Group	OMC					
	Total of all Isolates	Total Evaluable Isolates	Essential Agreement of Total	Essential Agreement of Evaluable	% Essential Agreement of Total	% Essential Agreement of Evaluable
M. abs. abs.	48	48	48	48	100.0%	100.0%
M. abs. bol.	4	4	4	4	100.0%	100.0%
M. abs. mass.	28	28	28	28	100.0%	100.0%
M. chelonae	35	35	34	34	97.1%	97.1%
M. fortiutum	35	35	35	35	100.0%	100.0%
M. immunogenum	20	20	20	20	100.0%	100.0%
M. mucogenicum	30	30	30	30	100.0%	100.0%
Total	200	200	199	199	99.5%	99.5%

Note: Five isolates exhibited insufficient growth and were excluded from final analysis.

Reproducibility

Reproducibility for OMC on the Sensititre susceptibility was 100%, with all 108 data points falling within ±1 $\log_2$ dilution of the modal MIC (**Table 4**). Reproducibility of TGC and DOX were 95.4% and 99.1% respectively.

Table 4. Reproducibility of OMC on the Sensititre Dried MIC Susceptibility System

OMC	Difference in the number of wells between test result and test mode						
	OFF-Scale	-2	-1	0	+1	+2	OFF-Scale
M. abs. abs. (erm I)				9			
M. abs. abs. (MAB30)			2	5	2		
M. abs. bol.				9			
M. abs. mass.				8	1		
M. abs. mass.				9			
M. chelonae				8	1		
M. fortuitum				9			
M. fortuitum			2	7			
M. immunogenum			4	5			
M. immunogenum			1	7	1		
M. mucogenicum			1	8			
M. mucogenicum				9			
TOTAL	0	0	10	93	5	0	0
Reproducibility Essential Agreement	108/108 = 100%						

MIC_{50} and MIC_{90} Values

The MIC_{50} represents the MIC at which ≥50% of the isolates are inhibited. The MIC_{90} represents the MIC at which ≥90% of the isolates are inhibited. MIC_{50} and MIC_{90} values were calculated for each organism and drug using the MICs obtained on the dried Sensititre plates after repeat testing (**Table 5**).

Results (cont.)

Table 5. Summary of MIC_{50} and MIC_{90} Values Obtained on Sensititre Dried Plates After Repeat Testing

Species	MIC Range (µg/ml)			MIC_{50}			MIC_{90}		
	OMC	TGC	DOX	OMC	TGC	DOX	OMC	TGC	DOX
M. abs. abs.	0.06-0.5	0.06-0.5	2->16	0.25	0.12	>16	0.5	0.5	>16
M. abs. bol.	0.12-0.5	0.03-0.25	2->16	0.12	0.06	2	0.5	0.25	>16
M. abs. mass.	0.06-1	0.03-0.5	1->16	0.5	0.25	>16	1	0.5	>16
M. chelonae	0.03-0.5	0.015-0.25	≤0.06 - >16	0.25	0.12	>16	0.5	0.25	>16
M. fortuitum	0.06-0.25	0.015-0.12	0.12->16	0.12	0.03	>16	0.12	0.12	>16
M. immunogenum	0.12-0.5	0.06-0.25	8->16	0.25	0.12	>16	0.5	0.25	>16
M. mucogenicum	0.12-2	0.03-0.25	≤0.06 - >16	0.5	0.06	4	1	0.25	>16
Combined	0.03-2	0.015-0.5	≤0.06 - >16	0.25	0.12	>16	0.5	0.25	>16

Conclusions

This study validates that the Sensititre susceptibility system demonstrates an equivalent level of performance compared to the CLSI M24 reference broth microdilution plate when testing OMC against rapidly growing non-tuberculous mycobacterial isolates. This study suggests that the Sensititre system is an acceptable method for susceptibility testing of OMC.

References

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