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

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ABSTRACT

Background: Cefiderocol (CFDC) (Shionogi & Co., Ltd., Osaka, Japan), is a novel parental siderophore cephalosporin displaying activity against highly resistant gram-negative non-fastidious organisms, including carbapenem-resistant strains of *Enterobacteriaceae* and non-fermenters. A multi-site evaluation was designed to determine the accuracy and reproducibility of CFDC susceptibility testing against gram-negative non-fastidious organisms using the Thermo Scientific™ Sensititre™ dried MIC susceptibility system compared with the CLSI (M07, M100)/ ISO 20776-1, ISO 20776-2 (CLSI/ISO) reference broth microdilution method (BMD). **Materials and Methods:** The Sensititre 18-24 hour MIC or Breakpoint Susceptibility System with CFDC in the dilution range of 0.03-64 µg/ml was tested against 374 recent clinical and challenge isolates and 11 reproducibility isolates. Microorganisms tested included 93 *Escherichia coli*, 168 *Klebsiella spp.*, 61 *Enterobacter spp.*, 50 *Citrobacter spp.*, 21 *Serratia marcescens*, 82 *Pseudomonas aeruginosa*, and 57 *Acinetobacter baumannii*. The Sensititre dried MIC susceptibility system was inoculated per manufacturers' instructions using cation adjusted Mueller Hinton broth (CAMHB). BMD was performed per CLIS/ISO guidelines using iron-depleted cation adjusted Mueller Hinton broth (ID-CAMHB). Recommended CLSI quality control (QC) organisms were tested daily and all results were within the published QC ranges. **Results:** Comparisons of the indicated gram-negative non-fastidious organisms MIC results on the Sensititre system to the CLIS/ISO BMD for automated and manual reads resulted in 94.0% and 95.0% essential agreement (EA; ±1 $\log_2$ dilution) for CFDC, respectively. Overall agreement for the reproducibility (±1 $\log_2$ dilution of the modal MIC) using automated and manual reads was 95% and 95.3%. **Conclusion:** The Sensititre susceptibility system demonstrates an equivalent level of performance compared to the CLSI/ISO BMD method when testing CFDC against non-fastidious 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 CFDC.
*** Abstract modified from original***

INTRODUCTION

Cefiderocol (Figure 1.) is the lone member of the siderophore cephalosporin class of antibiotics. This agent is transported across the outer membrane using active iron transporters which is enhanced in iron deficient conditions. CFDC has demonstrated *in vitro* activity and *in vivo* efficacy against multidrug-resistant (MDR) strains of gram-negative pathogens. This *in vitro* comparison study was done to validate the performance of cefiderocol on the commercially manufactured Thermo Scientific™ Sensititre™ 18 – 24 hour Dried Susceptibility Plate with the standard reference broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI M07/ M100) and ISO (20776-1). To establish equivalency for both auto and manual read methodologies, a series of studies were conducted at multiple trial sites including testing of clinical/challenge, reproducibility and quality control isolates.

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.
Cefiderocol was tested against: (Table 1.)

- 374 recent clinical gram-negative isolates across sites
- 147 challenge isolates at a single testing site
- 11 reproducibility isolates at each site (tested in triplicate over a 3 day testing period)
- 2 Quality Control Strains (ATCC) (Table 2.)

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 cefiderocol (0.03-64µg/ml). The dried plates were set up and tested by both automated and manual reading methodologies according to the manufacturer's instructions using CAMHB.
  - Reference broth microdilution plate was prepared and tested on each isolate according to the current Clinical Laboratory Standards Institute and ISO standard method.

Table 1. Organisms Tested	**Number Tested**
Clinical Isolates	374
CDC Challenge Isolates (one site)	147
Reproducibility Isolates (3 sites) (3 x day for 3 days)	11 (297)
ATCC Quality Control Strains	2 (124)
TOTAL	**942**

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 CFU, Sensititre 5X10^4-5X10^5 CFU

Table 2. Quality Control Strains	**CLSI QC Ranges** (µg/ml)
Escherichia coli ATCC 25922	0.06-0.5
Pseudomonas aeruginosa ATCC 27853	0.06-0.5

Results

Essential agreement for cefiderocol on the Sensititre susceptibility plate compared to the reference microdilution plate was calculated for each read method (Auto and Manual) using the ±1 $\log_2$ dilution standard. Essential agreement rates are shown for gram-negative non-fastidious isolates in **Tables 3 and 4**.

Clinical Isolates and Challenge Organisms

The overall essential agreement for cefiderocol within ±1 $\log_2$ dilution was **94.0%** for the auto read method and **95.0%** for the manual method.

Inter-laboratory Reproducibility

Reproducibility testing results for cefiderocol within ±1 $\log_2$ dilution from the modal MIC was **95%** for the auto read method and **95.3%** for the manual method.

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

The overall essential agreement for cefiderocol within +/- one $\log_2$ dilution, was 95.0% for the manual read method

Combined Total Isolates

Cefiderocol	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Escherichia coli	89	68	85	65	95.5%	95.6%
Klebsiella spp.	164	126	156	122	95.1%	96.8%
Enterobacter spp.	60	55	54	49	90.0%	89.1%
Citrobacter spp.	50	34	46	32	92.0%	94.1%
Serratia marcescens	21	21	20	20	95.2%	95.2%
P. aeruginosa	81	79	79	77	97.5%	97.5%
A. baumannii	56	52	55	51	98.2%	98.1%
Total	**521**	**435**	**495**	**416**	**95.0%**	**95.6%**

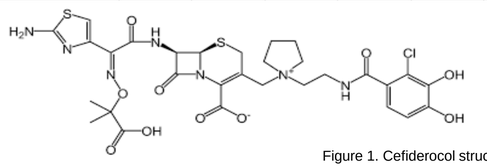

Figure 1. Cefiderocol structure

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

The overall essential agreement for cefiderocol within +/- one $\log_2$ dilution, was 94.0% for the auto read method

Combined Total Isolates

Cefiderocol	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Escherichia coli	89	68	85	65	95.5%	95.6%
Klebsiella spp.	164	124	153	117	93.3%	94.4%
Enterobacter spp.	60	55	54	49	90.0%	89.1%
Citrobacter spp.	50	34	46	32	92.0%	94.1%
Serratia marcescens	21	21	20	20	95.2%	95.2%
P. aeruginosa	81	80	79	78	97.5%	97.5%
A. baumannii	56	52	53	50	94.6%	96.2%
Total	**521**	**434**	**490**	**411**	**94.0%**	**94.7%**

RESULTS Cont.

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

Cefiderocol	**Auto Read**	**Manual Read**
Between-site total isolates tested	297	297
Between-site isolates within +/- 1 well from mode	281	283
Between-site reproducibility ratio	281	283
Between-site reproducibility %	**95%**	**95.3%**
Total essential agreement	281/297	283/297
Essential agreement %	**95%**	**95.3%**

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 and ISO 20776-1 reference broth microdilution plate when testing cefiderocol against gram-negative non-fastidious clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of cefiderocol.

REFERENCES

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