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P0284

A Multi-Site Study Comparing a Commercially Prepared Dried MIC Susceptibility System to the CLSI Broth Microdilution Method for Cefepime-Taniborbactam (formerly Cefepime/VNRX-5133) using Gram-Negative Non-Fastidious Organisms

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ABSTRACT

Background: Cefepime-taniborbactam (FTB; formerly cefepime/VNRX-5133) (Venatorx Pharmaceuticals, Malvern, PA) is an investigational combination of a fourth generation cephalosporin and a novel boronate-based beta-lactamase inhibitor (BLI) with *in vitro* activity against serine and metallo beta-lactamases. A 4-site evaluation was performed to determine the accuracy and reproducibility of FTB susceptibility testing against non-fastidious Gram-negative organisms using the Thermo Scientific™ Sensititre™ dried MIC susceptibility system (Sensititre system) compared with the CLSI (M07, M100)/ ISO 20776-1, ISO 20776-2 (CLSI/ISO) reference broth microdilution (BMD) method.
Materials/methods: The Sensititre system with FTB in the dilution range of 0.004/4-64/4 mg/L was used to test 734 recent clinical and challenge isolates and 10 reproducibility isolates. Microorganisms tested included 110 *E. coli*, 203 *Klebsiella* spp., 76 *Enterobacter cloacae*, 50 *Citrobacter* spp., 25 *S. marcescens,* 76 *Proteus* spp., 46 *Providencia* spp., 94 *M. morganii* and 54 *P. aeruginosa*. The Sensititre system was inoculated per manufacturer's instructions. BMD was performed per CLSI/ISO guidelines. Recommended CLSI quality control (QC) organisms were tested daily and all results were within the published QC ranges.
Results: FTB MIC results on the Sensititre system were comparable to those determined by CLSI/ISO BMD, with rates of essential agreement (EA; MICs ±1 $\log_2$ dilution) of 96.6% by autoread and 96.5% by manual read for *Enterobacteriaceae* and of 100% for both autoread and manual read for *P. aeruginosa*. Overall agreement for reproducibility (MICs ± 1 $\log_2$ dilution of the modal MIC) using autoread and manual read was 98.1% and 99.7%, respectively.
Conclusions: The Sensititre system demonstrated an equivalent level of performance compared to the CLSI/ISO BMD method when testing cefepime-taniborbactam against non-fastidious Gram-negative organisms. The high level of agreement obtained by the Sensititre system and the CLSI/ISO BMD method suggests that it is an acceptable method for susceptibility testing of FTB against Enterobacterales and *P. aeruginosa*.

INTRODUCTION

Taniborbactam (formerly VNRX-5133) is a novel investigational boronate-based beta-lactamase inhibitor (BLI) that is combined with cefepime for the treatment of infections due to Gram-negative non-fastidious organisms including those expressing extended-spectrum β-lactamases (ESBLs), *Klebsiella pneumoniae* carbapenemases (KPCs), oxacillinases (OXA), New Dehli metallo-beta-lactamases (NDM), and Verona integrin encoded metallo-beta-lactamases (VIM). This *in vitro* multi-site comparison study was done to evaluate the performance of cefepime-taniborbactam on the commercially manufactured Sensititre system, for both automated and manual reads, 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 4 lab clinical study was conducted, and the MIC results obtained using the Sensititre system were compared to the MIC results obtained from the CLSI M07 frozen reference plate.

MATERIALS AND METHODS

- The Sensititre system (Thermo Fisher Scientific, Oakwood Village, OH) is an *in vitro* diagnostic product for clinical susceptibility testing of both fastidious and non-fastidious organisms. Cefepime-taniborbactam was tested against: (Table 1.)
  - 556 recent clinical isolates across the four sites
  - 178 challenge isolates at a single testing site
  - 10 reproducibility isolates at each site (tested in triplicate over a three day period)
  - 5 Quality Control Strains (four ATCC ®, one NCTC)

MATERIALS AND METHODS Cont.

- Colony counts and purity plates were performed on the inocula of the Clinical, Challenge, Reproducibility and QC strains on each day of testing.
- Each isolate was tested using a:
  - Dried Sensititre susceptibility plate containing cefepime-taniborbactam (0.004/4-64/4µg/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. The reference plates were prepared and tested with each isolate according to the current Clinical Laboratory Standards Institute standard method.

Table 1. Organisms Tested	Number of Isolates (Number of Results)
Clinical Isolates (four sites)	556
CDC Challenge Isolates (one site)	178
Reproducibility Isolates (four sites) (3 x day for 3 days)	10 (360)
ATCC Quality Control Strains (minimum of 20 replicates of each strain at four sites)	5 (465)
TOTAL	**1559**

RESULTS

Quality Control

- Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges.
- Colony counts fell within expected ranges: reference plate 2-8X10^5 CFU/mL, Sensititre plate 5X10^4-5X10^5 CFU/mL

Table 2. Quality Control Strains	CLSI QC Ranges (µg/ml)
Escherichia coli ATCC® 25922™	0.03/4-0.12/4
Pseudomonas aeruginosa ATCC® 27853™	0.5/4-4/4
Escherichia coli ATCC® 35218™	0.016/4-0.06/4
Klebsiella pneumoniae ATCC® 700603™	0.12/4-0.5/4
Escherichia coli NCTC 13353	0.12/4-1/4

Essential agreement for cefepime-taniborbactam 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 Tables 3 and 4.

Clinical Isolates and Challenge Organisms

The overall essential agreement for cefepime-taniborbactam within ±1 $\log_2$ dilution was **96.5%** by manual method and **96.6%** by auto read method for *Enterobacterales* and **100%** by both methods for *Pseudomonas aeruginosa*.

Inter-laboratory Reproducibility

Reproducibility testing results for cefepime-taniborbactam within ±1 $\log_2$ dilution from the modal MIC was **97.8%** by auto read method and **99.7%** by manual read method.

RESULTS Cont.

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

The overall essential agreement for cefepime-taniborbactam within ± one $\log_2$ dilution, was 96.5% by manual read method for Enterobacterales and 100% for *Pseudomonas aeruginosa*

Combined Total Isolates

Cefepime-taniborbactam	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Citrobacter spp.	50	50	47	47	94.0%	94.0%
Enterobacter cloacae	76	74	73	71	96.1%	95.9%
Escherichia coli	110	110	110	110	100%	100%
Klebsiella spp.	203	202	197	196	97.0%	97.0%
Morganella morganii	94	94	90	90	95.7%	95.7%
Proteus spp.	76	76	73	73	96.1%	96.1%
Providencia spp.	46	46	45	45	97.8%	97.8%
Serratia marcescens	25	25	21	21	84.0%	84.0%
Enterobacterales Total	**680**	**677**	**656**	**653**	**96.5%**	**96.5%**
Pseudomonas aeruginosa	54	50	54	50	100%	100%

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

The overall essential agreement for cefepime-taniborbactam within ± one $\log_2$ dilution, was 96.6% by auto read method for Enterobacterales and 100% for *Pseudomonas aeruginosa*

Combined Total Isolates

Cefepime-taniborbactam	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Citrobacter spp.	50	50	47	47	94.0%	94.0%
Enterobacter cloacae	76	74	73	71	96.1%	95.9%
Escherichia coli	110	110	109	109	99.1%	99.1%
Klebsiella spp.	203	203	200	200	98.5%	98.5%
Morganella morganii	94	94	88	88	93.6%	93.6%
Proteus spp.	76	76	74	74	97.4%	97.4%
Providencia spp.	46	46	45	45	97.8%	97.8%
Serratia marcescens	25	25	21	21	84.0%	84.0%
Enterobacterales Total	**680**	**678**	**657**	**655**	**96.6%**	**96.6%**
Pseudomonas aeruginosa	54	51	54	51	100%	100%

RESULTS Cont.

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

Cefepime-taniborbactam	Auto Read	Manual Read
Between-site total isolates tested	360	360
Between-site isolates within +/- 1 well from mode	352	359
Between-site reproducibility %	**97.8%**	**99.7%**

CONCLUSIONS

This study validates that the Sensititre 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 cefepime-taniborbactam against Gram-negative non-fastidious clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of FTB against Enterobacterales and *Pseudomonas aeruginosa*.

REFERENCES

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