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

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ABSTRACT

Background: Ceftibuten-clavulanic acid (T/CA) (Achaogen, Inc. South San Francisco, CA) is a combination of a 3rd generation cephalosporin (ceftibuten) and a beta-lactamase inhibitor (clavulanic acid) displaying activity against highly resistant gram-negative non-fastidious organisms, including Extended Spectrum Beta Lactamase (ESBL) producing strains of *Enterobacteriaceae*. A 4-site evaluation was designed to determine the accuracy and reproducibility of T/CA susceptibility testing against non-fastidious gram negative 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 T/CA in the dilution range of 0.03/0.015-32/16µg/ml was tested against 455 recent clinical, challenge, and reproducibility *Enterobacteriaceae* isolates. The Sensititre dried MIC susceptibility system was inoculated per manufacturers' 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:** Comparisons of the indicated gram-negative non-fastidious organisms MIC results on the Sensititre system to the CLSI/ISO BMD for automated and manual reads resulted in 96.6% and 97.5% essential agreement (EA; +/- 1 $\log_2$ dilution) for T/CA, respectively. Overall agreement for the reproducibility (+/- 1 $\log_2$ dilution of the modal MIC) using automated and manual reads was 99.7% and 99.0% respectively. **Conclusion:** The Sensititre susceptibility system demonstrates an equivalent level of performance compared to the CLSI/ISO BMD method when testing T/CA against 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 T/CA.

INTRODUCTION

Ceftibuten-clavulanic acid is combination of ceftibuten, an approved third generation cephalosporin, and claulanate, an approved beta-lactamase inhibitor. This agent, T/CA, has demonstrated *in vitro* activity and *in vivo* efficacy against complicated urinary tract infections (cUTI) caused by *Escherichia coli* and *Klebsiella pneumoniae* that produce extended spectrum beta lactamase (ESBL). This *in vitro* comparison study was done to validate the performance of ceftibuten-clavulanic acid 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 4 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.
ceftibuten-clavulanic acid was tested against: (Table 1.)

- 367 recent clinical gram-negative isolates across the four sites
- 77 challenge isolates at a single testing site
- 11 reproducibility isolates at each site (tested in triplicate over a 3 day testing period)
- 4 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 ceftibuten-clavulanic acid (0.03/0.015-32/16µg/ml). The dried plates were set up and tested by both automated and manual reading methodologies according to the manufacturer's instructions.
  - 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 (4 sites)	367
Challenge Isolates (one site)	77
Reproducibility Isolates (4 sites) (3 x day for 3 days)	11 (396)
ATCC Quality Control Strains (at least 20 replicates of each strain at 3 sites, 1 site yielded fewer results)	4 (323)
TOTAL	**1163**

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	Expected CLSI QC Ranges (µg/ml)
Escherichia coli ATCC 25922	0.12/0.06-0.5/0.25
Escherichia coli NCTC 13353	0.25/0.12-1/0.5
Pseudomonas aeruginosa ATCC 27853	>32/16
Klebsiella pneumoniae ATCC 700603	0.06/0.03-0.25/0.12

Results

Essential agreement for ceftibuten-clavulanic acid 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 ceftibuten-clavulanic acid within ±1 $\log_2$ dilution was **96.6%** for the auto read method and **97.5%** for the manual method.

Inter-laboratory Reproducibility

Reproducibility testing results for ceftibuten-clavulanic acid within ±1 $\log_2$ dilution from the modal MIC was **99.7%** for the auto read method and **99.0%** 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 ceftibuten-clavulanic acid within +/- one $\log_2$ dilution, was 97.5% for the manual read method

Combined Total Isolates

Ceftibuten-clavulanic acid	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Escherichia coli	102	86	97	81	95.1%	94.2%
Klebsiella pneumoniae	94	59	90	56	95.7%	94.9%
Klebsiella oxytoca	43	9	43	9	100.0%	100.0%
Enterobacter cloacae	62	35	61	34	98.4%	97.1%
Klebsiella aerogenes	39	29	39	29	100.0%	100.0%
Citrobacter freundii	26	22	25	21	96.2%	95.5%
Serratia marcescens	26	24	26	24	100.0%	100.0%
Proteus vulgaris	23	1	23	1	100.0%	100.0%
Proteus mirabilis	29	3	29	3	100.0%	100.0%
Total	**444**	**268**	**433**	**258**	**97.5%**	**96.3%**

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 ceftibuten-clavulanic acid within +/- one $\log_2$ dilution, was 96.6% for the auto read method

Combined Total Isolates

Ceftibuten-clavulanic acid	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Escherichia coli	102	86	97	81	95.1%	94.2%
Klebsiella pneumoniae	94	59	89	55	94.7%	93.2%
Klebsiella oxytoca	43	8	43	8	100.0%	100.0%
Enterobacter cloacae	62	32	60	30	96.8%	93.8%
Klebsiella aerogenes	39	26	39	26	100.0%	100.0%
Citrobacter freundii	26	21	25	20	96.2%	95.2%
Serratia marcescens	26	23	25	23	96.2%	100.0%
Proteus vulgaris	23	1	22	1	95.7%	100.0%
Proteus mirabilis	29	3	29	3	100.0%	100.0%
Total	**444**	**259**	**429**	**247**	**96.6%**	**95.4%**

RESULTS Cont.

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

Ceftibuten-clavulanic acid	Auto Read	Manual Read
Between-site total isolates tested	396	396
Between-site isolates within +/- 1 well from mode	395	392
Between-site reproducibility ratio	395	392
Between-site reproducibility %	**99.7%**	**99.0%**
Total essential agreement	395/396	392/396
Essential agreement %	**99.7%**	**99.0%**

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 ceftibuten-clavulanic acid against gram-negative non-fastidious clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of ceftibuten-clavulanic acid.

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