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A Multi-Site Study of the Sensititre® Susceptibility System Compared with the CLSI Microdilution Method using New CLSI Breakpoints for MIC Determination of *Enterobacteriaceae* vs Cephems

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Abstract

Background: The Sensititre Susceptibility System can effectively be used for new CLSI breakpoints of cephems. An evaluation was performed to determine the accuracy and reproducibility of cefotaxime (FOT), ceftazidime (TAZ), and ceftriaxone (AXO) susceptibility testing using Sensititre 18-24h dry susceptibility plates (TREK Diagnostic Systems, Cleveland, OH) compared with the CLSI M100 reference broth microdilution method (BMD). Both Sensititre automated and manual reading methodologies were performed at three sites.

Methods: FOT, TAZ, and AXO (0.015-8µg/mL) were tested against 58 *Enterobacteriaceae* challenge isolates and 66 *Enterobacteriaceae* reproducibility isolates. Dried plates were inoculated as per manufacturers' instructions and BMD was performed per CLSI M7. CLSI quality control (QC) organisms were tested daily and all results were within CLSI QC ranges.

Results: Comparisons of cephem MIC results on the Sensititre system to the CLSI M100 BMD for both automated and manual reads resulted in 99% and 99% essential agreement (+/- one $\log_2$ dilution), respectively and 97% and 97% categorical agreement, respectively. With categorical agreements, there were no very major or major errors and ≤10% minor error rate for all isolates. The overall essential agreements (EA) and categorical agreements (CA) for the reproducibility (+/- one $\log_2$ dilution of the modal MIC) for automated and manual reads was 97%EA and 100% CA.

Conclusions: These study results for cephems indicate that the Sensititre 18-24h Susceptibility System for all challenge and reproducibility isolates gave reliable results using either the automated or manual read methods compared to the reference CLSI M7 BMD using the new CLSI M100 breakpoints.

Introduction

The CLSI subcommittee for antimicrobial susceptibility testing has been reviewing data from numerous *in vitro* clinical and PK/PD studies. In January 2010, it was decided to publish M100 standards referencing lower breakpoints for several cephalosporins and aztreonam for *Enterobacteriaceae*. A multi site comparison to evaluate the Sensititre 18-24h automated and manual susceptibility system to the CLSI microdilution method was performed using the recently published CLSI breakpoints for cephalosporins and aztreonam.

Materials & Methods

•Each isolate was tested using a Sensititre 18 – 24 susceptibility plate containing **cefotaxime**, **ceftazidime**, **ceftriaxone** and **aztreonam**. The dried plates were set-up and tested according to the manufacturers' instructions.

•The CLSI reference broth microdilution plate was prepared and tested on each isolate according to the Clinical Laboratory Standards Institute (CLSI M07and M100).

•Testing consisted of 58 Centers for Disease Control and Prevention (CDC) *Enterobacteriaceae* challenge isolates (Table 2 & 4).

•Reproducibility testing consisted of 22 *Enterobacteriaceae* isolates tested at all 3 sites on the Sensititre 18 – 24 hour susceptibility plate (Table 8).

•Quality control (QC) was assured by testing 20 replicates of each ATCC strain including *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, at each site on both the Sensititre test and reference method plates (Table 3).

•Colony counts were performed on the inoculum of the QC strains on each day of testing.

Table 1. Antimicrobials Tested

Antimicrobials Tested	Range Tested	Supplied by
Aztreonam	0.03-8µg/ml	Bristol Myers Squibb, Princeton, NJ
Cefotaxime	0.015-4µg/ml	Sigma-Aldrich, St. Louis, Missouri
Ceftazidime	0.03-4µg/ml	GlaxoSmithKline, Philadelphia, Pennsylvania
Ceftriaxone	0.015-8µg/ml	Sigma-Aldrich, St. Louis, Missouri

Table 2. Organisms Tested

Organisms Tested	Number Tested
CDC Challenge Isolates (one site)	
(58 *Enterobacteriaceae*)	58
Reproducibility Isolates (3 sites)	
(22 *Enterobacteriaceae*)	66
CLSI Quality Control Strains	
(20 replicates of each strain at 3 sites)	120

Table 3. Quality Control Strains

Aztreonam	CLSI MIC QC Ranges (µg/ml)
Escherichia coli ATCC 25922	0.06-0.25
Pseudomonas aeruginosa ATCC 27853	2-8
Cefotaxime	**CLSI MIC QC Ranges (µg/ml)**
Escherichia coli ATCC 25922	0.03-0.12
Pseudomonas aeruginosa ATCC 27853	8-32
Ceftazidime	**CLSI MIC QC Ranges (µg/ml)**
Escherichia coli ATCC 25922	0.06-0.5
Pseudomonas aeruginosa ATCC 27853	1-4
Ceftriaxone	**CLSI MIC QC Ranges (µg/ml)**
Escherichia coli ATCC 25922	0.03-0.12
Pseudomonas aeruginosa ATCC 27853	8-64

Table 4. Challenge Isolates Tested

Enterobacteriaceae	Number Tested
Aeromonas hydrophilia	2
Enterobacter aerogenes	5
Enterobacter agglomerans	1
Enterobacter cloacae	4
Escherichia coli	13
Klebsiella oxytoca	4
Klebsiella ozaenae	1
Klebsiella pneumoniae	5
Morganella morganii	1
Proteus mirabilis	3
Proteus vulgaris	3
Providencia rettgeri	3
Providencia stuartii	2
Serratia marcescens	10
Serratia odorifera	1
Total	**58**

Table 5. FDA MIC Interpretive Standard vs. New CLSI MIC Interpretive Standard Breakpoints

	FDA MIC Interpretive Standard (µg/ml)			New CLSI MIC Interpretive Standard (µg/ml)		
Aztreonam	**S**	**I**	**R**	**S**	**I**	**R**
	≤8	16	≥32	≤4	8	≥16
Cefotaxime	**FDA MIC Interpretive Standard (µg/ml)**			**New CLSI MIC Interpretive Standard (µg/ml)**		
	S	**I**	**R**	**S**	**I**	**R**
	≤8	16-32	≥64	≤1	2	≥4
Ceftazidime	**FDA MIC Interpretive Standard (µg/ml)**			**New CLSI MIC Interpretive Standard (µg/ml)**		
	S	**I**	**R**	**S**	**I**	**R**
	≤8	16	≥32	≤4	8	≥16
Ceftriaxone	**FDA MIC Interpretive Standard (µg/ml)**			**New CLSI MIC Interpretive Standard (µg/ml)**		
	S	**I**	**R**	**S**	**I**	**R**
	≤8	16-32	≥64	≤1	2	≥4

Results

Table 6. Summary Data and % Essential and Categorical Agreements of Aztreonam and CEPHEM Challenge Isolates

Auto Read Method with the New CLSI Breakpoints

Enterobacteriaceae	Total of All Isolates	Essential Agreement of Total	Categorical Agreement of Total	% Essential Agreement of Total	% Categorical Agreement of Total
Aztreonam	58	56	57	96.6%	98.3%
Cefotaxime	58	58	58	100.0%	100.0%
Ceftazidime	58	57	57	98.3%	98.3%
Ceftriaxone	58	57	54	98.3%	93.1%
Total	**232**	**228**	**226**	**98.3%**	**97.4%**

Table 7. Summary Data and % Essential and Categorical Agreements of Aztreonam and CEPHEM Challenge Isolates

Manual Read Method with the New CLSI Breakpoints

Enterobacteriaceae	Total of All Isolates	Essential Agreement of Total	Categorical Agreement of Total	% Essential Agreement of Total	% Categorical Agreement of Total
Aztreonam	58	55	57	94.8%	98.3%
Cefotaxime	58	58	56	100.0%	96.6%
Ceftazidime	58	57	58	98.3%	100.0%
Ceftriaxone	58	58	54	100.0%	93.1%
Total	**232**	**228**	**225**	**98.3%**	**97.0%**

Results

Essential agreement for the challenge isolates for **aztreonam**, **cefotaxime**, **ceftazidime**, and **ceftriaxone** were calculated using the +/- one $\log_2$ dilution standard for comparison studies. The categorical agreement was calculated using the recently published CLSI new interpretive breakpoints (Table 5). Essential agreement rates and categorical agreement rates are shown for *Enterobacteriaceae* in tables 6 and 7.

•Recommended CLSI quality control (QC) isolates were tested daily at all 3 sites and were within the CLSI expected QC ranges.

•Colony counts were performed and fell within the expected ranges for CLSI: 2 - $8x10^5$ and for Sensititre $5x10^4$ - $5x10^5$

CDC Challenge Organisms *Enterobacteriaceae*:

The agreement for **aztreonam** for auto/manual read was:
•Essential agreement 96.6%/94.8%
•Categorical agreement 98.3%/98.3%

The agreement for **cefotaxime** for auto/manual read was:
•Essential agreement 100%/100%
•Categorical agreement 100%/96.6%

The agreement for **ceftazidime** for auto/manual read was:
•Essential agreement 98.3%/98.3%
•Categorical agreement 98.3%/100%

The agreement for **ceftriaxone** for auto/manual read was:
•Essential agreement 98.3%/100%
•Categorical agreement 93.1%/93.1%

Interlaboratory Reproducibility *Enterobacteriaceae*:

Results for **aztreonam**, auto/manual read, within +/- one $\log_2$ dilution from the modal MIC were:
•Essential agreement 95%/95%
•Categorical agreement 100%/100%

Results for **cefotaxime**, auto/manual read, within +/- one $\log_2$ dilution from the modal MIC were:
•Essential agreement 98%/95%
•Categorical agreement 100%/100%

Results for **ceftazidime**, auto/manual read, within +/- one $\log_2$ dilution from the modal MIC were:
•Essential agreement 98%/100%
•Categorical agreement 100%/100%

Results for **ceftriaxone**, auto/manual read, within +/- one $\log_2$ dilution from the modal MIC were:
•Essential agreement 95%/97%
•Categorical agreement 100%/100%

Table 8. Interlaboratory Reproducibility % Essential & Categorical Agreements +/- one $\log_2$ Dilution of the Modal MIC with the New CLSI Breakpoints

	Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual
	Aztreonam		Cefotaxime		Ceftazidime		Ceftriaxone	
Between-site isolates tested	66	66	66	66	66	66	66	66
Between-site isolates within +/- 1 well from mode	63	63	65	63	65	66	63	64
Between-site reproducibility ratio	63/66	63/66	65/66	63/66	65/66	66/66	63/66	64/66
Between-site reproducibility %	95%	95%	98%	95%	98%	100%	95%	97%
Total Essential Agreement	63	63	65	63	65	66	63	64
Essential Agreement %	95%	95%	98%	95%	98%	100%	95%	97%
Total Categorical Agreement with New CLSI Breakpoints	66	66	66	66	66	66	66	66
Categorical Agreement % with New CLSI Breakpoints	100%	100%	100%	100%	100%	100%	100%	100%

Conclusions

This study validates that the Sensititre® 18 – 24 hour susceptibility system using the recently published new CLSI breakpoints demonstrates an equivalent level of performance compared to the CLSI M07 reference broth microdilution plate when testing **aztreonam**, **cefotaxime**, **ceftazidime**, and **ceftriaxone** against *Enterobacteriaceae* challenge and reproducibility isolates.

References

Clinical and Laboratory Standards Institute. **2009**. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Eighth Edition*. Approved document M07-A8. Wayne, PA: CLSI.

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