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A Multi-Site Study of the Sensititre® Susceptibility System Compared With the CLSI Microdilution Method for MIC Determination of Gram-Negative and Gram-Positive Organisms vs Ceftaroline

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Abstract

Background: Ceftaroline (CPT) (Forest Laboratories, Inc, New York, NY) is a novel, bactericidal, broad-spectrum injectable cephalosporin currently being developed for treatment of infections caused by gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Streptococcus pneumoniae*, and gram-negative bacteria. An evaluation was performed to determine the accuracy and reproducibility of the Sensititre® dried susceptibility system (TREK Diagnostic Systems, Cleveland, OH), using both automated and manual reading methodologies, compared with the CLSI M07 reference broth microdilution (BMD) method for gram-positive and gram-negative organisms vs CPT.

Materials and Methods: CPT (0.004-64 µg/mL) was tested against 1265 fresh clinical isolates, 225 challenge isolates, and 75 reproducibility isolates. These isolates consisted of the following: 116 Coag-negative *Staphylococcus* spp., 178 *S. aureus*, 114 *Enterococcus* spp., 327 *Streptococcus* spp. Beta-hemolytic Group, 187 *S. pneumoniae,* 135 *Streptococcus* spp. Viridans Group, 74 *E. coli*, 82 *Acinetobacter* spp., 66 *Klebsiella* spp., 31 *Citrobacter* spp., 19 *Providencia* spp., 44 *Enterobacter* spp., 53 *Proteus* spp., 17 *M. morganii*, 29 *Serratia* spp., 85 *Pseudomonas* spp., and 2 *A. hydrophila*. Dried plates were inoculated as per manufacturers' instructions, and the BMD was performed as per CLSI M07. Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI QC ranges.

Results: MIC results for CPT obtained with the Sensititre® susceptibility system compared with the CLSI M07 BMD demonstrated 97.8/98.3% essential agreement (±1 $\log_2$ dilution) for automated and manual read methods, respectively. Reproducibility was calculated as the percentage of results within ±1 $\log_2$ dilution of the modal MIC. Overall agreement for CPT reproducibility, AutoRead and manual read, was 97/97%, respectively.

Conclusions: Determination of CPT MICs for a broad collection of gram-positive and gram-negative organisms using the Sensititre® susceptibility system demonstrated essential equivalence to the reference BMD.

Introduction

Ceftaroline fosamil is a parenteral prodrug cephalosporin with excellent activity against resistant gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug-resistant *Streptococcus pneumoniae*, as well as gram-negative pathogens. Here we report results from a multi-site study conducted to evaluate the performance of **ceftaroline** on the Sensititre® 18-24 hour susceptibility system compared with that of the Clinical and Laboratory Standards Institute (CLSI) M07 reference broth microdilution method.

Materials and Methods

Susceptibility Testing Methods

- Indications for use: the Sensititre® 18-24 hour MIC or breakpoint susceptibility system is an in vitro diagnostic product for clinical susceptibility testing of fastidious and nonfastidious organisms
- Each isolate was tested using a Sensititre® 18-24 susceptibility plate containing **ceftaroline** (0.004-64 µg/mL; Forest Laboratories, Inc, New York, NY). Plates were prepared and tested according to the manufacturers' instructions
- The CLSI reference broth microdilution plate was prepared and tested according to CLSI M07 methods
- Testing consisted of 1265 fresh clinical isolates (combined 3 sites); approximately 133 gram-positive isolates, 152 *Streptococcus* spp., and 136 gram-negative isolates supplied by each site, and 225 Centers for Disease Control and Prevention (CDC) challenge isolates supplied to a single testing site (Tables 1 and 2)
- Reproducibility testing consisted of 25 gram-positive, 25 gram-negative, and 25 *Streptococcus* spp. isolates tested at all 3 sites on the Sensititre® 18-24 hour susceptibility plate (Table 1). The test plate results were compared with those from the CLSI reference broth microdilution plate
- Quality control (QC) was assured by testing 20 replicates of each ATCC strain, including *S. aureus* 29213, *Escherichia coli* 25922, and *S. pneumoniae* 49619, at each site (Tables 1 and 3)
- Colony counts were performed on the inoculum of the QC strains on each day of testing
- Recommended CLSI QC organisms were tested daily and were within the CLSI expected QC ranges (Table 3).

Table 1. Organisms Tested

Organisms tested	Number tested
Clinical isolates (combined 3 sites) (400 gram-positive, 457 *Streptococcus* spp., 408 gram-negative)	1265
CDC challenge isolates (1 site) (75 gram-positive, 75 *Streptococcus* spp., 75 gram-negative)	225
Reproducibility isolates (combined 3 sites) (25 gram-positive, 25 *Streptococcus* spp., 25 gram-negative)	75
CLSI quality control strains (20 replicates of each strain at 3 sites)	3 x 20

Table 2. Clinical and Challenge Isolates Tested

Gram-positive organisms	Number tested
Staphylococcus aureus	169
Coagulase-negative *Staphylococcus* spp.	111
Enterococcus spp.	108
Streptococcus spp. β-haemolytic Group (MHB)	87
Total	**475**
***Streptococcus* species**	**Number tested**
Streptococcus pneumoniae	177
Streptococcus spp. viridans group	130
Streptococcus spp. β-haemolytic group (MHB with LHB)	225
Total	**532**
Gram-negative organisms	**Number tested**
Enterobacteriaceae	316
Acinetobacter spp.	82
Other non-Enterobacteriaceae	85
Total	**483**

Table 3. Quality Control Strains

Quality control strains	CLSI MIC ranges (µg/mL)
Staphylococcus aureus ATCC 29213	0.12-0.5
Enterococcus faecalis ATCC 29212	0.008-0.03
Escherichia coli ATCC 25922	0.03-0.12
Streptococcus pneumoniae ATCC 49619	0.008-0.03

Results

Table 4. Summary Data and % Essential Agreement of Gram-positive Clinical and Challenge Isolates Using the Manual Read Method

Clinical isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase-negative *Staphylococcus* spp.	92	61	91	61	98.9	100.0
Staphylococcus aureus	142	141	142	141	100.0	100.0
Enterococcus spp.	91	58	91	58	100.0	100.0
Streptococcus spp. β-hemolytic group*	75	2	73	2	97.3	100.0
Total	**400**	**262**	**397**	**262**	**99.3**	**100.0**

Challenge isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase-negative *Staphylococcus* spp.	19	13	19	13	100.0	100.0
Staphylococcus aureus	27	27	27	27	100.0	100.0
Enterococcus spp.	17	16	17	16	100.0	100.0
Streptococcus spp. β-hemolytic group*	12	0	12	0	100.0	100.0
Total	**75**	**56**	**75**	**56**	**100.0**	**100.0**

Total isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase-negative *Staphylococcus* spp.	111	74	110	74	99.1	100.0
Staphylococcus aureus	169	168	169	168	100.0	100.0
Enterococcus spp.	108	74	108	74	100.0	100.0
Streptococcus spp. β-hemolytic group*	87	2	85	2	97.7	100.0
Total	**475**	**318**	**472**	**318**	**99.4**	**100.0**

**Streptococcus* spp. β-hemolytic group was tested with Mueller Hinton broth.

Table 5. Summary Data and % Essential Agreement of Gram-positive Clinical and Challenge Isolates Using the AutoRead Method

Clinical isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase-negative *Staphylococcus* spp.	90	59	90	59	100.0	100.0
Staphylococcus aureus	142	141	142	141	100.0	100.0
Enterococcus spp.	91	58	90	57	98.9	98.3
Streptococcus spp. β-hemolytic group*	75	2	73	2	97.3	100.0
Total	**398**	**260**	**395**	**259**	**99.2**	**99.6**

Challenge isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase-negative *Staphylococcus* spp.	19	12	19	12	100.0	100.0
Staphylococcus aureus	27	27	27	27	100.0	100.0
Enterococcus spp.	17	16	17	16	100.0	100.0
Streptococcus spp. β-hemolytic group*	12	0	12	0	100.0	100.0
Total	**75**	**55**	**75**	**55**	**100.0**	**100.0**

Total isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase-negative *Staphylococcus* spp.	109	71	109	71	99.1	100.0
Staphylococcus aureus	169	168	169	168	100.0	100.0
Enterococcus spp.	108	74	107	73	97.2	97.3
Streptococcus spp. β-hemolytic group*	87	2	85	2	97.7	100.0
Total	**473**	**315**	**470**	**314**	**99.4**	**99.7**

**Streptococcus* spp. β-hemolytic group was tested with Mueller Hinton broth.

Table 6. Summary Data and % Essential Agreement of *Streptococcus* spp. Clinical and Challenge Isolates Using the Manual Read Method

Clinical isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	152	96	146	95	96.1	100.0
Streptococcus spp. viridans group	105	96	103	94	98.1	97.9
Streptococcus spp. β-hemolytic group*	200	118	200	118	100.0	100.0
Total	**457**	**310**	**449**	**308**	**98.2**	**99.4**

Challenge isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	25	25	25	25	100.0	100.0
Streptococcus spp. viridans group	25	21	24	20	96.0	95.2
Streptococcus spp. β-hemolytic group*	25	12	25	12	100.0	100.0
Total	**75**	**58**	**74**	**57**	**98.7**	**98.3**

Total isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	177	121	171	121	96.6	100.0
Streptococcus spp. viridans group	130	117	127	114	97.7	97.4
Streptococcus spp. β-hemolytic group*	225	130	225	130	100.0	100.0
Total	**532**	**368**	**523**	**365**	**98.3**	**99.2**

**Streptococcus* spp. β-hemolytic group was tested with Mueller Hinton broth with lysed horse blood.

Table 7. Summary Data and % Essential Agreement of *Streptococcus* spp. Clinical and Challenge Isolates Using the AutoRead Method

Clinical isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	150	93	144	93	96.0	100.0
Streptococcus spp. viridans group	105	96	103	96	98.1	100.0
Streptococcus spp. β-hemolytic group*	200	118	196	114	98.0	96.6
Total	**455**	**307**	**443**	**303**	**97.4**	**98.7**

Challenge isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	25	25	25	25	100.0	100.0
Streptococcus spp. viridans group	25	20	24	20	96.0	100.0
Streptococcus spp. β-hemolytic group*	25	12	25	12	100.0	100.0
Total	**75**	**57**	**74**	**57**	**98.7**	**100.0**

Total isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	175	118	169	118	96.6	100.0
Streptococcus spp. viridans group	130	116	127	116	97.8	100.0
Streptococcus spp. β-hemolytic group*	225	130	221	126	98.2	96.9
Total	**530**	**364**	**517**	**360**	**97.5**	**98.9**

**Streptococcus* spp. β-hemolytic group was tested with Mueller Hinton broth with lysed horse blood.

Table 8. Summary Data and % Essential Agreement of Gram-negative Clinical and Challenge Isolates Using the Manual Read Method

Clinical isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Other non-Enterobacteriaceae	74	64	74	64	100.0	100.0
Enterobacteriaceae	258	216	258	216	100.0	100.0
Acinetobacter spp.	76	39	76	39	100.0	100.0
Total	**408**	**319**	**408**	**319**	**100.0**	**100.0**

Challenge isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Other non-Enterobacteriaceae	11	10	11	10	100.0	100.0
Enterobacteriaceae	58	47	56	46	96.6	97.9
Acinetobacter spp.	6	3	6	3	100.0	100.0
Total	**75**	**60**	**73**	**59**	**97.3**	**98.3**

Total isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Other non-Enterobacteriaceae	85	74	85	74	100.0	100.0
Enterobacteriaceae	316	263	314	262	99.4	99.6
Acinetobacter spp.	82	42	82	42	100.0	100.0
Total	**483**	**379**	**481**	**378**	**99.6**	**99.7**

Table 9. Summary Data and % Essential Agreement of Gram-negative Clinical and Challenge Isolates Using the AutoRead Method

Clinical isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Other non-Enterobacteriaceae	74	64	74	64	100.0	100.0
Enterobacteriaceae	258	216	257	216	99.6	100.0
Acinetobacter spp.	76	40	76	40	100.0	100.0
Total	**408**	**320**	**407**	**320**	**99.8**	**100.0**

Challenge isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Other non-Enterobacteriaceae	11	10	11	10	100.0	100.0
Enterobacteriaceae	58	47	54	44	93.1	93.6
Acinetobacter spp.	6	3	6	3	100.0	100.0
Total	**75**	**60**	**71**	**57**	**94.7**	**95.0**

Total isolates

	Number of isolates		Essential agreement		% Essential agreement	
Organism group	All	Evaluable	Total	Evaluable	Total	Evaluable
Other non-Enterobacteriaceae	85	74	85	74	100.0	100.0
Enterobacteriaceae	316	263	311	260	98.1	98.4
Acinetobacter spp.	82	43	82	43	100.0	100.0
Total	**483**	**380**	**478**	**377**	**99.0**	**99.2**

Essential agreement for the clinical and challenge isolates combined was calculated for each method (AutoRead and manual read) using the ±1 $\log_2$ dilution standard. The calculations for Evaluable excluded any test results where the MICs were off-scale for the dilutions tested. Essential agreement rates are shown for gram-positive (Tables 4 and 5), *Streptococcus* spp. (Tables 6 and 7), and gram-negative (Tables 8 and 9) isolates.

Clinical Isolates and CDC Challenge Organisms

- **Gram-Positive Isolates:** Overall essential agreement for **ceftaroline**, within ±1 $\log_2$ dilution, was 99.4% for the manual and 99.4% for the AutoRead method (Tables 4 and 5)
- ***Streptococcus* spp.:** Overall essential agreement for **ceftaroline**, within ±1 $\log_2$ dilution, was 98.3% for the manual and 97.5% for the AutoRead method (Tables 6 and 7)
- **Gram-Negative Isolates:** Overall essential agreement for **ceftaroline**, within ±1 $\log_2$ dilution, was 99.6% for the manual and 99% for the AutoRead method (Tables 8 and 9).

Interlaboratory Reproducibility

- **Gram-Positive and Gram-Negative Isolates:** Reproducibility testing results, within ±1 $\log_2$ dilution from the expected result, were 100% for both the manual and AutoRead methods (Table 10)
- ***Streptococcus* spp.:** Reproducibility testing results, within ±1 $\log_2$ dilution from the expected result, were 99% for both the manual and AutoRead methods (Table 10).

Table 10. Interlaboratory Reproducibility % Essential Agreements ±1 $\log_2$ Dilution of the Modal MIC for Ceftaroline

	Auto gram-positive	Manual gram-positive	Auto *Streptococcus* spp.	Manual *Streptococcus* spp.	Auto gram-negative	Manual gram-negative
Between-site total isolates tested	75	75	75	75	75	75
Between-site isolates within ± 1 well from mode	75	75	74	74	75	75
Between-site reproducibility ratio	75/75	75/75	74/75	74/75	75/75	75/75
Between-site reproducibility %	100	100	99	99	100	100
Total essential agreement	75	75	74	74	74	74
Essential agreement %	100	100	99	99	99	99

Conclusions

This study validates that the Sensititre® 18-24 hour susceptibility system (both AutoRead and manual read) demonstrated an equivalent level of performance compared with that of the CLSI M07 reference broth microdilution plate when testing **ceftaroline** against gram-positive, *Streptococcus* spp., and gram-negative clinical and challenge isolates. The high level of essential agreement obtained with the Sensititre® 18-24 hour susceptibility method suggests that this is an acceptable method for susceptibility testing of **ceftaroline**.

References

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