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An Equivalency Study of the Sensititre® Dried MIC System Compared With the CLSI Broth Dilution Reference Method for Ceftaroline and Comparator Antimicrobials

C. Bastulli,[1] N. Holliday,[1] C.C. Knapp,[1] S.B. Killian,[1] D. Biek,[2] J. Ge[2]

[1]TREK Diagnostic Systems, Cleveland, Ohio, [2]Cerexa Inc., Alameda, California

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Cara M. Bastulli
Research Scientist
TREK Diagnostic Systems
982 Keynote Circle, Suite 6
Cleveland, Ohio 44131
cbastulli@trekds.com
216-351-8735 x:172 phone
216-351-5456 fax

Amended Abstract

Background: Ceftaroline (CPT) is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Streptococcus pneumoniae* (MDRSP), as well as common gram-negative pathogens. CPT is currently in phase III development. An evaluation was undertaken to determine the accuracy and reproducibility of the Sensititre 18-24 hour dried susceptibility system (manufactured by TREK Diagnostic Systems, Cleveland, OH) with CPT compared to the Clinical and Laboratory Standards Institute (CLSI) M7 reference broth microdilution (BMD) method. CPT was tested over the range of 0.004 – 64 µg/mL.

Materials and Methods: 200 isolates (36 *S pneumoniae,* 22 Groups A and B streptococci, 10 *S aureus,* 4 *Staphylococcus epidermidis,* 1 *Staphylococcus saprophyticus,* 8 *Enterococcus faecalis*, 23 *Haemophilus influenzae,* 23 *Escherichia coli,* 11 *Enterobacter aerogenes,* 1 *Enterobacter agglomerans,* 11 *Enterobacter cloacae,* 10 *Proteus mirabilis,* 5 *Proteus vulgaris,* 4 *Serratia marcescens,* 24 *Klebsiella pneumoniae,* 4 *Klebsiella oxytoca,* 1 *Morganella morganii,* 1 *Providencia rettgeri,* 1 *Providencia stuartii*) were tested with CPT and 7 comparators using the Sensititre 18-24 hour dried susceptibility plate and CLSI M7 BMD for comparison. For reproducibility testing, 10 isolates were tested 3 times daily over a period of 3 days. Recommended CLSI quality-control (QC) organisms were tested daily and were within the CLSI expected QC ranges for all comparators.

Results: Comparison of the Sensititre plate to the CLSI M7 BMD resulted in 100% agreement (± 1 $\log_2$ dilution) for CPT and > 99% for comparators. Reproducibility was calculated as the percentage of results within ± 1 $\log_2$ dilution of the modal value. Overall agreement for the reproducibility of CPT and for comparators was 100%.

Conclusions: This evaluation indicates that the Sensititre 18-24 hour dried susceptibility plate with CPT yields highly reproducible results equivalent to the CLSI M7 BMD. The Sensititre plate is an acceptable method for susceptibility testing of CPT.

Introduction and Purpose

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* (MDRSP), as well as common gram-negative pathogens. In plasma, ceftaroline fosamil is quickly converted into the bioactive ceftaroline (CPT), which exhibits bactericidal activity. Susceptibility test methods, including broth microdilution assay, have been evaluated and validated for active CPT according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The quality-control (QC) limits for CPT were established for broth microdilution assay using frozen susceptibility plate. The successful development of a dried susceptibility plate will provide a reliable and convenient testing tool for clinical microbiology laboratories in determining the susceptibility of CPT against causative pathogens.

Here we report results from a series of studies conducted to determine the accuracy and reproducibility of the Sensititre 18-24 hour dried susceptibility system with CPT compared with the CLSI M7 reference broth microdilution (BMD) method.

Materials and Methods

- Sensititre 18-24 hour dried susceptibility system and CLSI M7 BMD (TREK Diagnostic Systems, Cleveland, OH)
- 200 clinical and challenge isolates consisting of:
  - 36 *Streptococcus pneumoniae*
  - 22 Groups A and B β-hemolytic streptococci
  - 10 *Staphylococcus aureus*
  - 4 *Staphylococcus epidermidis*
  - 1 *Staphylococcus saprophyticus*
  - 8 *Enterococcus faecalis*
  - 23 *Haemophilus influenzae*
  - 23 *Escherichia coli*
  - 11 *Enterobacter aerogenes*
  - 1 *Enterobacter agglomerans*
  - 11 *Enterobacter cloacae*
  - 10 *Proteus mirabilis*
  - 5 *Proteus vulgaris*
  - 4 *Serratia marcescens*
  - 24 *Klebsiella pneumoniae*
  - 4 *Klebsiella oxytoca*
  - 1 *Morganella morganii*
  - 1 *Providencia rettgeri*
  - 1 *Providencia stuartii*
- Isolates were tested with CPT and seven comparators (Table 1) using the Sensititre 18-24 hour dried susceptibility plate and by CLSI M7 BMD for comparison

Table 1. The Eight Tested Antimicrobials With Test Ranges

Antimicrobial	Range Tested, µg/mL	Supplied By
Ceftaroline	0.004-64	Cerexa (Alameda, CA)
Ceftriaxone	0.015-64	Sigma (St. Louis, MO)
Ceftazidime	0.06-64	GlaxoSmithKline (Philadelphia, PA)
Ampicillin	0.03-32	Sigma (St. Louis, MO)
Amikacin	0.25-64	Sigma (St. Louis, MO)
Imipenem	0.015-16	Merck (Whitehouse Station, NJ)
Levofloxacin	0.008-16	Johnson & Johnson (Spring House, PA)
Vancomycin	0.06-64	Amresco (Solon, OH)

- Daily QC isolates were run on both the reference and dried plate:
  - *S aureus* ATCC 29213
  - *E coli* ATCC 25922
  - *E faecalis* ATCC 29212
  - *S pneumoniae* ATCC 49619
  - *H influenzae* ATCC 49247
  - *H influenzae* ATCC 49766
- All 200 isolates were tested on frozen and dried plates. Dried plates were tested according to manufacturer instructions, and frozen reference plates were tested according to CLSI M7
- Reproducibility: 10 strains, including the CLSI, ATCC, and QC isolates, were tested in triplicate on 3 separate days. This testing generated 90 values that did not demonstrate greater than ± 1 well variation (≤ 10%)
- Repeat testing was performed for any organism/antimicrobial combination that resulted in greater than ± 1 $\log_2$ dilution error. These were repeated in triplicate on both the dried test plate and the reference plate
- Standard ATCC QC strains for broth susceptibility testing were set up daily, as per isolates being tested on the test day. By completion of the study, at least 20 replicates were run of each appropriate QC strain.

Results

S aureus

MIC Distribution for Clinical Isolates vs CPT

Ceftaroline Results — *S aureus* — Sensititre Test Results (rows) vs *S aureus* CLSI Reference Results (columns)

MIC	≤ 0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	> 64
≤ 0.004																
0.008																
0.015																
0.03																
0.06																
0.12						4										
0.25						2	4									
0.5																
1																
2																
4																
8																
16																
32																
64																
> 64																
Totals	0	0	0	0	0	6	4	0	0	0	0	0	0	0	0	0

H influenzae

MIC Distribution for Clinical Isolates vs CPT

Ceftaroline Results — *H influenzae* — Sensititre Test Results (rows) vs *H influenzae* CLSI Reference Results (columns)

MIC	≤ 0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	> 64
≤ 0.004	1															
0.008	6	3														
0.015		8	2													
0.03			3													
0.06																
0.12																
0.25																
0.5																
1																
2																
4																
8																
16																
32																
64																
> 64																
Totals	7	11	5	0	0	0	0	0	0	0	0	0	0	0	0	0

S pneumoniae

MIC Distribution for Clinical Isolates vs CPT

Ceftaroline Results — *S pneumoniae* — Sensititre Test Results (rows) vs *S pneumoniae* CLSI Reference Results (columns)

MIC	≤ 0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	> 64
≤ 0.004	4	8														
0.008		2	1													
0.015			2	1												
0.03				3	2											
0.06					3	2										
0.12						4	2									
0.25							1									
0.5									1							
1																
2																
4																
8																
16																
32																
64																
> 64																
Totals	4	10	3	4	5	6	3	0	1	0	0	0	0	0	0	0

E coli

MIC Distribution for Clinical Isolates vs CPT

Ceftaroline Results — *E coli* — Sensititre Test Results (rows) vs *E coli* CLSI Reference Results (columns)

MIC	≤ 0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	> 64
≤ 0.004																
0.008																
0.015																
0.03																
0.06				3	1											
0.12					3	4										
0.25						2	5									
0.5							2	1								
1																
2																
4																
8												2				
16																
32																
64																
> 64																
Totals	0	0	0	3	4	6	7	1	0	0	0	2	0	0	0	0

Essential agreements for all 200 isolates were calculated using the ±1 $\log_2$ dilution standard for comparison (Table 2). Reproducibility results were also calculated using the ±1 $\log_2$ dilution of the CLSI reference broth microdilution plate (Tables 3, 4, and 5).

Table 2. Essential Agreement For 200 Isolates Within ± 1 $\log_2$ Dilution

Antimicrobial	% Essential Agreement (Before/After Repeat Testing)
Ceftaroline	98.8/100
Ceftriaxone	98.1/100
Ceftazidime	100/100
Ampicillin	99.8/99.8
Amikacin	100/100
Imipenem	99.5/100
Levofloxacin	100/100
Vancomycin	99.6/100

Table 3. Reproducibility Results for CPT and Comparator Antimicrobials Within ± 1 $\log_2$ Dilution

Antimicrobial	Number of Isolates Tested	% Essential Agreement
Ceftaroline	10	100
Ceftriaxone	10	100
Ceftazidime	10	100
Ampicillin	10	100
Amikacin	10	100
Imipenem	10	100
Levofloxacin	10	100
Vancomycin	10	100

Table 4. Reproducibility: Sensititre Test Results for CPT in Triplicate on 3 Days

Reproducibility	Sensititre Test Results CPT									MODE
	Day 1			Day 2			Day 3			
Organism	A	B	C	A	B	C	A	B	C	
S aureus	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
E coli	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
H influenzae	0.06	0.12	0.06	0.06	0.06	0.06	0.12	0.12	0.12	0.06
S pneumoniae	0.03	0.03	0.03	0.03	0.015	0.015	0.015	0.03	0.03	0.03
S aureus	0.12	0.25	0.12	0.25	0.25	0.25	0.25	0.25	0.25	0.25
S epidermidis	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.5	0.5	0.5
E coli	0.25	0.25	0.25	0.5	0.5	0.5	0.25	0.5	0.25	0.25
H influenzae	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
S pneumoniae	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Group B β hemolytic streptococci	0.03	0.015	0.015	0.015	0.015	0.03	0.015	0.015	0.015	0.015

% Essential agreement for total = 100% (± 1 well of the modal value).

Table 5. Reproducibility: CLSI Reference Results for CPT in Triplicate on 3 Days

Reproducibility	CLSI Reference Results CPT									MODE
	Day 1			Day 2			Day 3			
Organism	A	B	C	A	B	C	A	B	C	
S aureus	0.12	0.12	0.12	0.12	0.12	0.12	0.25	0.12	0.25	0.12
E coli	0.06	0.06	0.06	0.12	0.06	0.12	0.06	0.06	0.06	0.06
H influenzae	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
S pneumoniae	0.015	0.015	0.015	0.015	0.03	0.03	0.015	0.015	0.015	0.015
S aureus	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
S epidermidis	0.25	0.12	0.12	0.25	0.25	0.25	0.25	0.25	0.25	0.25
E coli	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
H influenzae	0.008	0.008	0.008	0.008	0.008	0.008	0.008	≤0.004	0.008	0.008
S pneumoniae	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Group B β hemolytic streptococci	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015

% Essential agreement for total = 100% (± 1 well of the modal value).

Conclusions

- The Sensititre 18-24 hour dried susceptibility plate yielded highly reproducible results equivalent to the CLSI M7 reference broth microdilution method for susceptibility testing of ceftaroline
- The overall essential agreement for ceftaroline and > 99% of comparators, within a ± 1 $\log_2$ dilution range, was 100% for the Sensititre plate and the CLSI M7 broth microdilution method
- Intralaboratory reproducibility was 100% for ceftaroline and all comparator drugs
- Susceptibility data derived from the dried plate for QC strains were in agreement with the QC limits approved by CLSI for ceftaroline
- This study validates that the Sensititre 18-24 hour dried susceptibility plate is an acceptable method for susceptibility testing of ceftaroline.

References

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