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Accuracy of Sensititre Dry-Form Broth Microdilution Panels to Determine Ceftaroline-Avibactam MIC Values

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Abstract

Background: Avibactam (AVI), a β-lactamase inhibitor, has been combined with ceftaroline (CPT; an anti-MRSA cephalosporin) to expand activity against Gram-negative (GN) bacilli. To allow early testing in clinical trials and surveillance protocols, validation of dry-form broth microdilution (BMD) commercial panels (Sensititre; ST) with extended shelf lives was performed. Here we present a single reference laboratory comparison study of ST versus reference frozen-form BMD MIC results.

Methods: CPT-AVI was tested over a 0.008/4 to 16/4 µg/ml MIC range in BMD panels (reference and ST). Reference BMD was performed by CLSI M07-A9 method using Mueller-Hinton broth with appropriate supplements, and endpoints read by an automated method (ST only but not *H. influenzae* [HI]) as well as manually. QC used several appropriate ATCC strains having CLSI ranges; all results were within limits. 525 strains (240 GN; and 285 Gram-positive) were processed and analyzed for variations between ST and CLSI MICs with essential agreement (EA; ± one doubling dilution) targeted at ≥95%. Key tested pathogens within CPT indications were: *S. aureus* (110), pneumococcus (30), other streptococci (85), Enterobacteriaceae (115) and HI (85). Intra-laboratory reproducibility was assessed (25 strains in triplicate).

Results: Table shows MIC distribution comparisons for both methods and two analysis sets (all and on-scale [O-S] results). Gram-positive ST MIC/reference MIC ratios were 1 for >60% of strains and EA was ≥99.5%; only one enterococcus at a ratio of 4 was unacceptable. GN strains showed 73.8% with MIC comparisons at a ratio of 1, 99.2% EA and only two Enterobacteriaceae with a ratio of 4. Enterococci, some streptococci and enteric bacilli showed skewing (28.6-67.9%) of ST CPT-AVI MICs toward higher values (O-S results). Overall, EA was 99.3-99.4%. Intra-laboratory agreement was 100.0% ± one doubling dilution step. Automated endpoints were equivalent.

Conclusions: CPT-AVI MIC results from the ST panel (dry-form) was essentially the same as results from frozen-form reference BMD test values; >99.0% EA without significant skewing across 11 pathogen groups. These reproducible/validated results for a commercial system can be applied during conclusion of clinical trials and post-regulatory approval of the CPT-AVI combination.

Abstract Table

Organisms or Groups (no. tested)	Sensititre MIC/Reference MIC ratio (occurrences): All comparisons 0.25	0.5	1	2	4	On-scale (O-S) comparisons[a] 0.25	0.5	1	2	4
Gram-positive species (285)	0	13	187	84	1	0	12	128	62	1
Gram-negative species (240)	0	16	177	45	2	0	13	83	40	2
All strains (525)	0	29	364	129	3	0	25	211	102	3

a. Only results having MIC values for both methods not at the extremes of the dilution schedules
b. Includes: MRSA (53), *S. lugdunensis* (10), *S. haemolyticus* (10), *E. faecalis* (10; 3 VRE) and *E. faecium* (10; 7 VRE), *S. pyogenes* (30), *S. agalactiae* (30) and five viridans group species

Introduction

Ceftaroline-avibactam is a combination of the antibacterial ceftaroline and the novel non β-lactam β-lactamase inhibitor avibactam. Avibactam does not have intrinsic antibacterial activity; however, it does inhibit Class A, C and some Class D β-lactamases. When avibactam is combined with an active β-lactam agent, such as ceftaroline, its ability to inhibit β-lactamases protects the activity of the β-lactam from enzyme degradation.

Ceftaroline fosamil, the prodrug of active ceftaroline, is a cephalosporin approved by the United States Food and Drug Administration (USA-FDA) and European Medicines Agency (EMA). Ceftaroline has broad-spectrum anti-bacterial in vitro activity against resistant Gram-positive organisms, including methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant (MDR) strains of *Streptococcus pneumoniae.* Ceftaroline also has activity against Enterobacteriaceae; however, it is not active against extended-spectrum β-lactamase (ESBL) phenotype strains. Adding avibactam to ceftaroline, markedly expands the activity to include ESBL and cephalosporinase producing phenotype strains.

To address the intermediate needs for a reliable in vitro susceptibility testing device for ceftaroline-avibactam following regulatory approval, a single reference laboratory study results are presented for a validation of the ThermoFisher Scientific (Sensititre®) dry-form MIC product. This system was compared to the reference CLSI (2012) frozen-form method results, read manually and by an automated device.

Methods

A systematic method development and validation study was designed to compare the Sensititre® dry-form broth microdilution panel results monitoring ceftaroline-avibactam (MIC range, ≤0.008/4 to 16/4 µg/ml) to those results derived from reference CLSI (2012) M07-A9 frozen-form panels. Endpoints read manually and by automated commercially available devices were also compared. All tests were performed in standardized cation-adjusted Mueller-Hinton broth with appropriate supplements (HTM or 2.5-5% lysed horse blood) for testing fastidious species. Study design followed guidelines found in CLSI M23-A3 (2008), FDA guidances and those previously used by our research group.

The study examined 525 recent clinical and challenge isolates including Gram-positive (285) and -negative (240) organisms in 11 pathogen groups. The following organisms were tested: *Staphylococcus aureus* (110; 53 MRSA), coagulase-negative staphylococci (CoNS; 20 including 10 *S. lugdunensis,* 10 *S. haemolyticus*), enterococci (40; 20 *E. faecalis,* 20 *E. faecium* with 10 being VRE), β-streptococci (60; two species), *Streptococcus pneumoniae* (30), 25 other streptococci (five species) and 240 Gram-negative isolates (see Table 2). Endpoints were only read manually for *H. influenzae* (85 strains) see manually read results displayed in Table 2. Quality control (QC) used multiple ATCC strains (29212, 29213, 25922, 27853, 49247 35218, 700603 and 49619); all QC results were within published CLSI (2014) ranges. Reproducibility with three replicates across numerous species groups (25 strains) was also determined. Target essential agreement (EA) between methods was ± one doubling dilution at ≥95% for compared MIC results (Table 2).

Results

- Table 1 is reproduced from a recent publication from our laboratories (Flamm, Farrell, Sader and Jones, 2014) comparing the spectrum for ceftaroline combined with avibactam when tested against nearly 15,000 Enterobacteriaceae and Gram-positive cocci cultured from cutaneous infections
- Against enteric bacilli, the susceptibility rates for ceftaroline at ≤0.5 µg/ml (CLSI breakpoint) were markedly increased to 98.3-100.0% when combined with 4 µg/ml of avibactam, except for *S. marcescens* (84.2% susceptible, see Table 1). Similarly, *S. aureus* (CLSI breakpoint at ≤1 µg/ml) had ceftaroline-avibactam susceptibility rates at 99.4% and was very potent against the streptococci (MIC_{90}, 0.03-0.06 µg/ml)
- To assure an accurate recognition of this enhanced ceftaroline-avibactam activity by a commercial device, 525 pathogens were tested and compared to the reference CLSI (2012) MIC method results (Table 2)
  - Comparisons between methods were analyzed using all data (525 data points) and only those having on-scale MIC results (341) for both methods; results were similar with an overall EA of 99.1-99.4%
  - Among the 285 Gram-positive cocci, 65.6% of Sensititre® MIC values for ceftaroline-avibactam were identical to those of the reference MIC test, and all results showed a 99.6% EA
  - Enterobacteriaceae and *H. influenzae* (manual reads only) ceftaroline-avibactam MIC comparisons showed great agreement of Sensititre® results with those of the reference method (76.0%). All Gram-negative species showed a slight trend toward higher Sensititre® MIC values with 30.8% of comparison MICs at a ≥two-fold greater value (Table 2, "on-scale comparisons")
  - Automated endpoints did not significantly differ from manually read MIC results (data not shown)
- Organisms (only three) outside of EA limits were enterococci (one) and enteric bacilli (two); only 0.6% overall (Table 2). Intra-laboratory reproducibility was within ± one doubling dilution for all (100.0%) 25 triplicate comparisons (data not shown).

Table 2. Comparative ceftaroline-avibactam MIC values obtained from a ThermoFisher Scientific (Sensititre®) broth microdilution dry-form panel and the reference CLSI method using 525 clinical and challenge strains

Organisms or Groups (no. tested)	Sensititre MIC/Reference MIC ratio (occurrences): All comparisons 0.25	0.5	1	2	4	On-scale comparisons[a] 0.25	0.5	1	2	4
Gram-positive species (285)	0	13	187	84	1	0	12	128	62	1
S. aureus (110)[b]	0	8	84	18	0	0	8	84	18	0
CoNS (20)[c]	0	4	15	1	0	0	4	15	1	0
Enterococci (40)[d]	0	0	20	19	1	0	0	9	18	1
S. pneumoniae (30)	0	0	20	10	0	0	0	3	5	0
S. pyogenes (30)	0	1	28	1	0	0	0	0	0	0
S. agalactiae (30)	0	0	15	15	0	0	0	15	11	0
Other streptococci (25)[e]	0	0	5	20	0	0	0	2	9	0
Gram-negative species (240)	0	16	177	45	2	0	13	83	40	2
Enterobacteriaceae (115)[f]	0	12	71	30	2	0	12	68	30	2
P. aeruginosa (20)	0	0	13	7	0	0	0	11	6	0
Acinetobacter spp. (10)	0	1	3	6	0	0	1	1	4	0
H. influenzae (85)	0	3	81	1	0	0	0	3	0	0
M. catarrhalis (10)	0	0	9	1	0	0	0	0	0	0
All strains (525)	0	29	364	129	3	0	25	211	102	3

a. On-scale = only results having MIC values for both methods not at the extremes of the dilution schedules were tabulated
b. Includes: MRSA (53 strains)
c. Includes: *S. lugdunensis* (10 strains) and *S. haemolyticus* (10 strains)
d. Includes: *E. faecalis* (10 strains; three vancomycin-resistant) and *E. faecium* (10 strains; seven vancomycin-resistant)
e. Includes five species
f. Includes 13 species

Table 1. Summary of ceftaroline-avibactam activity tested against bacterial isolates from patients with skin and skin structure infections in the USA (2010-2012)[a]

Organism[a]	No. of Isolates	No. of isolates (cumulative %) inhibited at ceftaroline-avibactam MIC (µg/ml): ≤0.03	0.06	0.12	0.25	0.5	1	2	4	MIC_{50}	MIC_{90}
Staphylococcus aureus	8,422	5 (0.1)	35 (0.5)	622 (7.9)	3438 (48.7)	3431 (89.4)	843 (99.4)	48 (100.0)	--	0.5	1
MSSA	4,089	5 (0.1)	35 (1.0)	620 (16.1)	3328 (97.5)	101 (100.0)	--	--	--	0.25	0.25
MRSA	4,333	--	--	2 (0.0)	110 (2.6)	3330 (79.4)	843 (98.9)	48 (100.0)	--	0.5	1
Coagulase-negative staphylococci	622	52 (8.4)	133 (29.7)	101 (46.0)	252 (86.5)	77 (98.9)	6 (99.8)	1 (100.0)	--	0.25	0.5
β-hemolytic streptococci	1,523	1,512 (99.3)	11 (100.0)	--	--	--	--	--	--	≤0.03	0.03
Streptococcus pyogenes	706	706 (100.0)	--	--	--	--	--	--	--	≤0.03	0.03
Streptococcus agalactiae	671	669 (99.7)	2 (100.0)	--	--	--	--	--	--	≤0.03	0.03
Other streptococci	146	137 (93.8)	9 (100.0)							0.03	0.03
Viridans group streptococci	411	353 (85.9)	37 (94.9)	6 (96.4)	6 (97.8)	6 (99.3)	3 (100.0)	--	--	0.03	0.06
Escherichia coli	923	687 (74.4)	201 (96.2)	28 (99.2)	3 (99.6)	4 (100.0)	--	--	--	0.03	0.06
ESBL-screen negative-phenotype	805	635 (78.9)	160 (98.8)	10 (100.0)	--	--	--	--	--	0.03	0.06
ESBL-screen positive-phenotype	118	52 (44.1)	41 (78.8)	18 (94.1)	3 (96.6)	4 (100.0)	--	--	--	0.06	0.12
Meropenem-susceptible (MIC, ≤1 µg/ml)	922	687 (74.5)	200 (96.2)	28 (99.2)	3 (99.6)	4 (100.0)	--	--	--	≤0.03	0.06
Meropenem-non-susceptible (MIC, ≥2 µg/ml)	1	--	1 (100.0)	--	--	--	--	--	--	--	--
Klebsiella pneumoniae	641	146 (22.8)	319 (72.5)	94 (87.2)	46 (94.4)	26 (98.4)	6 (99.4)	2 (99.7)	2 (100.0)	0.06	0.25
ESBL-screen negative-phenotype	543	139 (25.6)	305 (81.8)	65 (93.7)	26 (98.5)	8 (100.0)	--	--	--	0.06	0.12
ESBL-screen positive-phenotype	98	7 (7.1)	14 (21.4)	29 (51.0)	20 (71.4)	18 (89.8)	6 (95.9)	2 (98.0)	2 (100.0)	0.12	1
Meropenem-susceptible (MIC, ≤1 µg/ml)	598	145 (24.2)	319 (77.6)	86 (92.0)	35 (97.8)	13 (100.0)	--	--	--	0.06	0.12
Meropenem-non-susceptible (MIC, ≥2 µg/ml)	43	1 (2.3)	0 (2.3)	8 (20.9)	11 (46.5)	13 (76.7)	6 (90.7)	2 (95.3)	2 (100.0)	0.5	1
Klebsiella oxytoca	281	149 (53.0)	99 (88.3)	22 (96.1)	6 (98.2)	4 (99.6)	1 (100.0)	--	--	0.03	0.12
Enterobacter spp.	599	65 (10.9)	172 (39.6)	237 (79.1)	79 (92.3)	36 (98.3)	10 (100.0)	--	--	0.12	0.25
Citrobacter spp.	208	59 (28.4)	107 (79.8)	33 (95.7)	7 (99.0)	1 (99.5)	1 (100.0)	--	--	0.06	0.12
Proteus mirabilis	413	49 (11.9)	244 (70.9)	102 (95.6)	14 (99.0)	2 (99.5)	2 (100.0)	--	--	0.06	0.12
Morganella morganii	239	137 (57.3)	66 (84.9)	21 (93.7)	11 (98.3)	3 (99.6)	1 (100.0)	--	--	0.03	0.12
Serratia marcescens	222	1 (0.5)	1 (0.9)	27 (13.1)	62 (41.0)	96 (84.2)	32 (98.6)	1 (99.1)	2 (100.0)	0.5	1

a. From Flamm, Farrell, Sader and Jones (2014)

Conclusions

- Sensititre® ceftaroline-avibactam dry-form broth microdilution MIC panels demonstrated excellent (EA at 99.6%) validation results with the CLSI reference frozen-form panel MIC values, regardless of manual or automated endpoint reading or whether the tested organisms were Gram-positive or -negative pathogens
- These single-laboratory Sensititre® validation study findings confirmed via a FDA 510 K-style study, appear to allow accurate determination of ceftazidime-avibactam MIC values by clinical laboratories following this combination's regulatory approval. This broad-spectrum antimicrobial will be welcomed by physicians to address therapy of infections caused by MDR ESKAPE pathogens among the Enterobacteriaceae, as well as methicillin-resistant staphylococci and various MDR streptococcal species.

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