Validation of Sensititre Dry-Form Broth Microdilution Panels for Susceptibility Testing of Ceftazidime-Avibactam, a Broad-Spectrum- β -Lactamase Inhibitor Combination

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Ceftazidime-avibactam is a broad-spectrum- β -lactamase inhibitor combination in late-stage clinical development for the treatment of serious infections. In preparation for clinical microbiology laboratory use, a validation experiment was initiated to evaluate a commercial broth microdilution product (Sensititre dried MIC susceptibility system) compared to reference panels using 525 recent clinical isolates. Among 11 pathogen groups, all had Sensititre MIC/reference MIC ratios predominantly at 1 (47.5% to 97.5%), and automated and manual endpoint results did not differ. *Enterobacteriaceae* MIC comparisons showed a modest skewing of Sensititre MIC results toward an elevated MIC (33.9%), but the essential agreement was 98.9% with 100.0% reproducibility. In conclusion, Sensititre panels produced accurate ceftazidime-avibactam MIC results, allowing quality MIC guidance for therapy following regulatory approvals.

Ceftazidime-avibactam consists of a broad-spectrum β -lactam antimicrobial agent (ceftazidime) in combination with the non- β -lactam- β -lactamase inhibitor avibactam (1). This combination has activity against Gram-negative bacteria producing Ambler class A, class C, and some class D β -lactamases (2–5) (Table 1). Avibactam is very potent and inactivates β -lactamase enzymes very efficiently, with low 50% inhibitory concentrations (IC₅₀s), thus generating a stable enzyme-avibactam product against commonly occurring enzymes (TEM-1, CTX-M-15) (1, 4–6). The role of avibactam in the combination is to protect ceftazidime from destruction by a variety of serine β -lactamases, thus allowing clinical success in clinical trials (7, 8). The *in vitro* spectrum of ceftazidime-avibactam activity includes *Enterobacteriaceae* producing extended-spectrum β -lactamases (ESBLs) and nonmetallo-carbapenemases (*Klebsiella pneumoniae* carbapenemase and some OXA enzymes) (2–5). Ceftazidime-avibactam has also been shown to be active against *Pseudomonas aeruginosa* strains containing a derepressed AmpC enzyme but would not be active against strains resistant to ceftazidime or some carbapenems due to efflux pump mechanisms (1).

To become an effective therapeutic agent against emerging multidrug-resistant (MDR) pathogens such as the ESCAPE organisms (*Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* species) (9), laboratories must be able to accurately test the combination to guide therapy (7, 8). In this report, we describe validation study results from a commercial method (Sensititre dried MIC susceptibility system; Thermo Fisher Scientific, Cleveland, OH, USA) developed for ceftazidime-avibactam susceptibility testing compared to the reference broth microdilution method of the Clinical and Laboratory Standards Institute (CLSI) (10). (This study was presented as poster 2542 at the 114th General Meeting of the American Society for Microbiology, Boston, MA, 2014 [11].)

A systematic method development and validation study was designed (12–14) to compare the Sensititre panel MIC results for ceftazidime-avibactam (MIC range, ≤ 0.015 to 32 $\mu\text{g/ml}$ of ceftazidime with a fixed 4 $\mu\text{g/ml}$ concentration of avibactam) to those MIC values derived from the CLSI frozen-form panel (10). End-

points read manually and by the automated commercially available device were tabulated. All tests were performed in standardized cation-adjusted Mueller-Hinton broth with appropriate supplements (HTM or 2.5% to 5% lysed horse blood) for testing fastidious species (10–15).

We examined 525 Gram-positive ($n = 285$) and Gram-negative ($n = 240$) isolates from 11 pathogen groups recently cultured from samples of patients in the United States. The following organisms were tested: *S. aureus* ($n = 110$, 55 of which were methicillin resistant), coagulase-negative staphylococci (CoNS; $n = 20$, consisting of 10 *Staphylococcus lugdunensis* and 10 *Staphylococcus haemolyticus* isolates), enterococci ($n = 40$; 20 of these were *Enterococcus faecalis* isolates, 3 of which were vancomycin resistant [VRE], and 20 were *E. faecium* isolates, 10 of which were VRE), β -hemolytic streptococci ($n = 60$; 2 species), *Streptococcus pneumoniae* ($n = 30$), other streptococci ($n = 25$; 5 species), and 240 Gram-negative isolates (Table 2). Endpoints were only read manually for the *Haemophilus influenzae* isolates ($n = 85$ strains). Multiple ATCC strains (29212, 29213, 25922, 27853, 49247, 35218, 49619, and 700603) were used for quality control (QC); all QC results were within published CLSI ranges (15). Reproducibility with three replicates for three testing events across several species groups (25 strains) was also determined. The target essential agreement (EA) between methods was ± 1 doubling dilution at $\geq 95\%$ for all compared MIC results.

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Table 1 is presented from a recent U.S. resistance surveillance publication by Flamm et al. (2) comparing the spectrums for ceftazidime tested alone and combined with avibactam when tested against 5,605 *Enterobacteriaceae* and 1,259 *P. aeruginosa* isolates. Against the enteric bacilli, the susceptibility rates (85.5% to 91.8%) for ceftazidime at ≤ 4 $\mu\text{g/ml}$ (15, 16) were increased significantly to 99.4% to 100.0% when combined with a fixed 4- $\mu\text{g/ml}$ concentration of avibactam. However, the ≤ 4 $\mu\text{g/ml}$ breakpoint applied to ceftazidime was based on a dosing regimen of 1 g every 8 h (q8h), whereas the ceftazidime-avibactam regimen uses 2 g q8h that would cover a breakpoint at ≥ 8 $\mu\text{g/ml}$ based on the probability of joint target attainment from pharmacokinetic/pharmacodynamic modeling (15, 16). Therefore, ceftazidime-avibactam coverage of the *Enterobacteriaceae* isolates overall was 99.7% to 100.0% at ≤ 8 $\mu\text{g/ml}$ (Table 1). *P. aeruginosa* had ceftazidime-alone susceptibility rates of 79.5% to 89.7% that were markedly expanded to 95.8% to 98.7% with the inclusion of avibactam 500 mg q8h (breakpoint, ≤ 8 $\mu\text{g/ml}$) (Table 2).

To ensure an accurate recognition of this enhanced ceftazidime-avibactam activity by a commercial device, 525 pathogens were tested and compared to reference MIC method results (Table 2). Comparisons between methods were analyzed using all MIC data (525 data points) as well as using only those with on-scale (OS) MIC results for both test methods. The two comparative analyses of results were similar, with an overall essential agreement of 98.9%. Among the 285 Gram-positive cocci, 78.6% of Sensititre MIC values were identical to those of the reference MIC test. *Enterobacteriaceae* and *H. influenzae* (manual reads only) MIC comparisons showed a slight skewing of Sensititre results toward a higher ceftazidime-avibactam MIC result, but other Gram-negative species showed excellent concordance. Finally, the automated endpoints did not differ from manually read MIC results (data not shown).

Organisms ($n = 6$) outside EA limits were *Enterococcus* (1 isolate), streptococci (2 isolates), enteric bacillus (1 isolate), and *H. influenzae* (2 isolates), i.e., only 1.1% of compared strains (Table 2). Intralaboratory reproducibility was within ± 1 doubling dilution for all (100.0%) 25 strains (225 total comparisons).

Sensititre ceftazidime-avibactam broth microdilution MIC panels demonstrated an excellent validation agreement (EA, 98.9%) compared to reference frozen-form panel MIC results, regardless of manual or automated endpoint reading and regardless of whether the organism is a Gram-positive or -negative species (Table 2). These single-laboratory Sensititre study results confirmed in a validation-style study design appear to allow accurate determination of ceftazidime-avibactam MIC values by clinical microbiology laboratories following Sensititre regulatory approval. Ceftazidime-avibactam activity and spectrum tested against Gram-negative pathogens in the United States (2) markedly increased ceftazidime coverage to 99.7% to 100.0% and 95.8% to 98.7% for *Enterobacteriaceae* and *P. aeruginosa*, respectively, across recent surveillance strains from four different infection types (bacteremia, pneumonia, intraabdominal infection, urinary tract infection) (Table 1). This very-broad-spectrum- β -lactamase inhibitor combination (1–6) should be welcomed by physicians to address therapy of infections caused by contemporary MDR Gram-negative pathogens (10).

TABLE 1 Summary of ceftazidime-avibactam and ceftazidime (alone) activity when tested against selected Gram-negative bacterial isolates from patients in U.S. medical centers (2012)^a

Organism	Infection type (no. tested) ^b	Antimicrobial agent	No. (cumulative %) of isolates inhibited at ceftazidime-avibactam MIC (µg/ml) of:											MIC ₅₀	MIC ₉₀
			≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	≥32		
<i>Enterobacteriaceae</i>	BSI (1,269)	CAZ-AVt ^c	121 (11.7)	464 (48.2)	450 (83.7)	137 (94.5)	53 (98.7)	12 (99.6)	3 (99.8)	0 (99.8)	1 (99.9)	1 (100.0)		0.12	0.25
		Ceftazidime	26 (2.0)	201 (17.9)	428 (51.6)	295 (74.9)	111 (83.6)	31 (86.1)	15 (87.2)	9 (87.9)	22 (89.7)	36 (92.5)	34 (100.0)	0.12	16
	Pneumonia (1,738)	CAZ-AVt ^c	125 (8.9)	460 (35.3)	682 (74.6)	280 (90.7)	100 (96.4)	44 (99.0)	6 (99.3)	1 (99.4)	6 (99.7)	3 (99.9)	2 (100.0)	0.12	0.25
		Ceftazidime	48 (2.8)	241 (16.6)	514 (46.2)	409 (69.7)	185 (80.4)	45 (83.0)	27 (84.5)	17 (85.5)	24 (86.9)	33 (88.8)	195 (100.0)	0.25	32
	IAI (410)	CAZ-AVt ^c	36 (11.5)	122 (41.2)	157 (79.5)	43 (90.0)	28 (96.8)	10 (99.3)	3 (100.0)					0.12	0.25
UTI (2,188)		Ceftazidime	12 (2.9)	46 (14.1)	143 (49.0)	91 (71.2)	38 (80.5)	17 (84.6)	4 (85.6)	2 (86.1)	6 (87.6)	7 (89.3)	44 (100.0)	0.25	32
		CAZ-AVt ^c	309 (17.0)	771 (52.3)	731 (85.7)	208 (95.2)	75 (98.6)	22 (99.6)	3 (99.8)	4 (100.0)	1 (100.0)			0.06	0.25
		Ceftazidime	79 (3.6)	405 (22.1)	774 (57.5)	492 (80.0)	154 (87.0)	53 (89.4)	34 (91.0)	18 (91.8)	23 (92.9)	29 (94.2)	127 (100.0)	0.12	2
<i>P. aeruginosa</i>	BSI (141)	CAZ-AVt ^c				1 (0.7)	4 (3.5)	56 (43.3)	46 (75.9)	18 (88.7)	11 (96.5)	1 (97.2)	4 (100.0)	2	8
		Ceftazidime					2 (1.4)	13 (10.6)	70 (60.3)	25 (78.0)	7 (83.0)	2 (84.4)	22 (100.0)	2	>32
	Pneumonia (881)	CAZ-AVt ^c	3 (0.3)	2 (0.6)	15 (2.3)		59 (9.0)	312 (44.4)	265 (74.5)	132 (89.4)	56 (95.8)	25 (98.6)	12 (100.0)	2	8
		Ceftazidime	1 (0.1)	2 (0.3)	2 (0.6)		30 (4.0)	143 (20.2)	328 (57.4)	133 (72.5)	61 (79.5)	45 (84.6)	136 (100.0)	2	32
	IAI (82)	CAZ-AVt ^c						35 (42.7)	30 (79.3)	11 (92.7)	3 (96.3)	2 (98.8)	1 (100.0)	2	4
UTI (155)		Ceftazidime					11 (13.4)	39 (61.0)	12 (75.6)	8 (85.4)	1 (86.6)	11 (100.0)	2	32	
		CAZ-AVt ^c					9 (5.8)	47 (36.1)	63 (76.8)	23 (91.6)	11 (98.7)	1 (99.4)	1 (100.0)	2	4
		Ceftazidime					5 (3.2)	18 (14.8)	70 (60.0)	30 (79.4)	16 (89.7)	6 (93.5)	10 (100.0)	2	16

^a Reproduced from reference 2.

^b BSI, bloodstream infections; LAI, intraabdominal infections; UTI, urinary tract infections.

^c CAZ-AVIF, ceftazidime-avibactam. Ceftazidime MIC measured in the presence of a fixed concentration of avibactam 4 $\mu\text{g/ml}$.

TABLE 2 Comparison of the ceftazidime-avibactam combination MIC results for Sensititre (Thermo Fisher Scientific) and the reference broth microdilution method (CLSI) in 525 tested clinical isolates

Organism or group (no. tested)	No. of occurrences at indicated candidate MIC/reference MIC ratio									
	All comparisons (<i>n</i> = 525)					On-scale comparisons (<i>n</i> = 416) ^a				
	0.25	0.5	1	2	4	0.25	0.5	1	2	4
Gram-positive species (285)										
<i>S. aureus</i> (110) ^b	0	7	86	17	0	0	7	68	13	0
CoNS (20) ^c	0	6	14	0	0	0	6	11	0	0
<i>Enterococci</i> (40) ^d	0	0	39	0	1 ^e	0	0	0	0	0
<i>S. pneumoniae</i> (30)	0	4	25	1	0	0	4	25	1	0
<i>Streptococcus pyogenes</i> (30)	0	0	21	9	0	0	0	21	9	0
<i>Streptococcus agalactiae</i> (30)	0	0	25	5	0	0	0	25	5	0
Other streptococci (25) ^f	0	1	14	8	2	0	1	14	8	2
Gram-negative species (240)										
<i>Enterobacteriaceae</i> (115) ^g	0	20	55	39	1	0	18	55	37	1
<i>P. aeruginosa</i> (20)	0	6	12	2	0	0	6	12	1	0
<i>Acinetobacter</i> spp. (10)	0	1	7	2	0	0	1	6	2	0
<i>H. influenzae</i> (85)	0	9	44	30	2	0	7	32	11	0
<i>Moraxella catarrhalis</i> (10)	0	1	9	0	0	0	0	7	0	0
All strains (525)	0	55	351	113	6	0	50	276	87	3

^a MIC results were on the dilution schedule for both compared methods.^b Includes 53 strains of methicillin-resistant *S. aureus* (MRSA).^c Includes the following coagulase-negative staphylococci (CoNS): *S. lugdunensis* (10 strains) and *S. haemolyticus* (10 strains).^d Includes *E. faecalis* (10 strains; 3 were vancomycin resistant [VRE]) and *E. faecium* (10 strains; 7 were VRE).^e One strain had a ratio of ≥ 16 .^f Includes 5 species groups.^g Includes 13 species.

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