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In Vitro Antibacterial Activity of the Ceftazidime-Avibactam Combination against *Enterobacteriaceae*, Including Strains with Well-Characterized β-Lactamases

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The novel β-lactamase inhibitor avibactam is a potent inhibitor of class A, class C, and some class D enzymes. The *in vitro* antibacterial activity of the ceftazidime-avibactam combination was determined for a collection of *Enterobacteriaceae* clinical isolates; this collection was enriched for resistant strains, including strains with characterized serine β-lactamases. The inhibitor was added either at fixed weight ratios to ceftazidime or at fixed concentrations, with the latter type of combination consistently resulting in greater potentiation of antibacterial activity. In the presence of 4 μg/ml of avibactam, the ceftazidime MIC_{50} and MIC_{90} (0.25 and 2 μg/ml, respectively) were both below the CLSI breakpoint for ceftazidime. Further comparisons with reference antimicrobial agents were performed using this fixed inhibitor concentration. Against most ceftazidime-susceptible and -nonsusceptible isolates, the addition of avibactam resulted in a significant increase in ceftazidime activity, with MICs generally reduced 256-fold for extended-spectrum β-lactamase (ESBL) producers, 8- to 32-fold for CTX-M producers, and >128-fold for KPC producers. Overall, MICs of a ceftazidime-avibactam combination were significantly lower than those of the comparators piperacillin-tazobactam, cefotaxime, ceftriaxone, and cefepime and similar or superior to those of imipenem.

Antimicrobial resistance is a growing problem among Gram-negative pathogens worldwide. The β-lactams, which have been among the most widely used of antimicrobial agents, have increasingly been compromised by resistance, primarily through the expression of β-lactamases in Gram-negative organisms. Of particular concern are enzymes able to target the expanded-spectrum β-lactams, including the AmpC enzymes (Ambler class C cephalosporinases), the so-called extended-spectrum β-lactamases (ESBLs), and the carbapenemases.

The addition of a β-lactamase inhibitor to cephalosporins, monobactams, or penicillins is a valuable alternative to carbapenems for the treatment of infections with the most commonly isolated Gram-negative pathogens, which usually express one or several Ambler class A or C β-lactamases. However, the inhibitors currently used in the clinical setting (clavulanate, tazobactam, and sulbactam) have an inhibition spectrum that only partially covers the clinically relevant enzymes.

Avibactam is the first of a new class of non-β-lactam β-lactamase inhibitors referred to as diazabicyclooctanes (1). Avibactam displays potent, broad-spectrum inhibition of Ambler class A and class C β-lactamases, as well as some class D enzymes. More significantly, avibactam shows effective inhibition of clinically important enzymes, such as ESBLs (including the CTX-M subclass), KPC, AmpC, and some OXA enzymes. The inhibitor has little intrinsic antibacterial activity but efficiently protects β-lactams from hydrolysis in bacterial strains producing β-lactamases (2–6). The pharmacokinetic profile of avibactam is similar to that of ceftazidime (1), and a combination of 2 g ceftazidime and 500 mg avibactam is currently in phase 3 clinical evaluation (http://clinicaltrials.gov).

The purpose of this study was to evaluate the antimicrobial activity of ceftazidime-avibactam combinations against a large selection of clinical isolates of *Enterobacteriaceae*, including strains with well-characterized β-lactamases. The level of protection conferred by the inhibitor was evaluated using various amounts of avibactam, either at a fixed concentration or at a fixed weight/weight ratio with ceftazidime. In addition, some marketed antimicrobial agents were tested as comparators.

MATERIALS AND METHODS

Bacterial isolates. A total of 166 *Enterobacteriaceae* isolates (45 *Escherichia coli*, 45 *Klebsiella* sp., 15 *Citrobacter freundii*, 15 *Enterobacter* sp., 11 *Morganella morganii*, 19 *Providencia* sp., 13 *Proteus* sp., and 3 *Serratia marcescens* isolates) were tested. This collection consisted of clinical isolates, primarily of European and U.S. origin, originating from a number of sources. Three additional strains (*E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 7000603, and *Enterobacter cloacae* P99) were included as internal standards. Many of the isolates and all of the ceftazidime-resistant strains contained well-characterized β-lactamases of class A, C, or D. Strains with a ceftazidime MIC at or below the lowest concentration of ceftazidime tested (0.125 μg/ml) were excluded from the study, as were any strains containing class B metallo-β-lactamases.

Antimicrobial susceptibility testing. MIC values were determined using the reference broth microdilution method with cation-adjusted Mueller-Hinton broth (Becton-Dickinson, France) as described by the

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TABLE 1 Inhibition of strains by ceftazidime and avibactam

Antibiotic[a] (ratio or AVI concn)	Cumulative % of strains with MIC (μg/ml) (n = 169)										
	≤0.125	0.25	0.5	1	2	4	8	16	32	64	128
CAZ	0	9	22	30	40	44	49	54	60	68	75
CAZ-AVI (1:1)	8	18	35	60	77	91	98	99	100	100	100
CAZ-AVI (2:1)	6	17	33	57	73	86	96	99	100	100	100
CAZ-AVI (4:1)	5	15	30	50	67	79	93	99	99	100	100
CAZ-AVI (8:1)	5	13	28	46	63	75	88	96	99	100	100
CAZ-AVI (2 μg/ml)	23	41	62	77	82	91	95	98	98	99	99
CAZ-AVI (4 μg/ml)	28	53	69	85	91	97	99	99	99	99	100
CAZ-AVI (8 μg/ml)	57	74	87	94	99	99	99	99	99	100	100

[a] CAZ, ceftazidime; AVI, avibactam.

Clinical and Laboratory Standards Institute (CLSI) (7). Bacterial inocula were adjusted to contain 5×10^5 CFU/ml. MIC values were interpreted according to breakpoints established by CLSI guidelines (8). Since a breakpoint has not yet been set for ceftazidime-avibactam, the approved ceftazidime breakpoint was used for this combination (≤8 μg/ml). The following commercially available antimicrobials were used: ceftazidime, piperacillin, ceftriaxone, cefotaxime, cefepime, and imipenem. The β-lactamase inhibitor tazobactam was assessed at a fixed concentration of 4 μg/ml. Avibactam was tested either at fixed concentrations of 2, 4, and 8 μg/ml or at fixed ceftazidime-avibactam weight/weight ratios of 1/1, 2/1, 4/1, and 8/1.

Compounds. Ceftazidime pentahydrate was supplied by Sandoz (Kundl, Austria). Imipenem and cefepime were obtained from USP (Rockville, MA), and cefotaxime, ceftriaxone, piperacillin, and tazobactam were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Avibactam was synthesized by Novexel (Romainville, France).

Determination of β-lactamases. Detection and characterization of β-lactamases expressed in various bacterial strains were achieved by *bla* gene detection, eventually combined with isoelectric focusing (IEF) of cell extracts.

Gene detection. Bacterial DNAs were subjected to PCR amplification profiling using appropriate primers for detection of the most common *bla* gene families, as follows: class A, TEM, SHV, VEB, PER, GES, CARB, CTX-M, and KPC families; class C, CMY-1/MOX, CMY-2, DHA, ACC, ACT-1, and FOX plasmidic subgroups; and class D, OXA-1, OXA-2, OXA-10/13, and OXA-18/45 families.

PCRs were performed using Ready-To-Go reagents (GE Healthcare) according to the manufacturer's instructions. Briefly, cell lysis was performed at 99°C for 3 min, followed by 30 cycles of amplification (94°C for 1 min, 55°C for 1 min, and 72°C for 1 min) and a final extension at 72°C for 5 min.

RESULTS AND DISCUSSION

The isolates used were collected from a number of sources, and many were included in the study because they are ceftazidime resistant and/or possess a variety of well-characterized β-lactamases. Eighty-two isolates were ceftazidime susceptible, and 87 were ceftazidime resistant. Because the collection was enriched with ceftazidime-resistant isolates, it is likely that some species showed an atypical susceptibility distribution.

Activities of differing ceftazidime-avibactam combinations. The activities of the different combinations of ceftazidime-avibactam are summarized in Table 1. All combinations showed improvements in activity over that of ceftazidime alone. The MIC_{50}/MIC_{90} for fixed weight/weight combinations of ceftazidime-avibactam ranged from 2/16 μg/ml for the 8:1 combination to 1/4 μg/ml for the 1:1 combination. Fixed concentrations of 2, 4, and 8 μg/ml avibactam gave consistently lower MICs than those of the fixed ratio combinations, with MIC_{50}/MIC_{90} values of 0.5/4, 0.25/2, and ≤0.125/1 μg/ml, respectively.

Ceftazidime-avibactam and comparators against *Enterobacteriaceae* species. Table 2 shows summary MIC data by species for ceftazidime-avibactam and a range of comparator compounds. In this table, only data for the CLSI-approved ceftazidime-avibactam combination for *in vitro* antibacterial activity testing (the ceftazidime MIC in the presence of a fixed concentration [4 μg/ml] of avibactam) are reported. Table 3 shows some of the same data summarized by enzyme type.

A total of 164/169 isolates showed a reduced ceftazidime MIC when ceftazidime was combined with 4 μg/ml avibactam, with improvements in the MIC_{50}/MIC_{90} ratio against all species. A collection of ceftazidime-resistant strains would be expected to be broadly resistant to other cephalosporins, and this was the case in the present study. The MIC_{90}s of piperacillin-tazobactam and the cephalosporins cefotaxime, ceftriaxone, and cefepime were generally significantly higher than those of ceftazidime-avibactam, except against the *Proteeae* (see below). The comparator with the broadest spectrum was the carbapenem imipenem. But even in this case, ceftazidime-avibactam MIC_{90}s were lower for the majority of species.

Against *E. coli*, the ceftazidime MIC_{50}/MIC_{90} ratio was reduced substantially in the presence of avibactam, and 45/46 isolates had MICs of ≤4 μg/ml for the ceftazidime-avibactam combination. The one resistant isolate contained the class A VEB-1 enzyme, against which avibactam has poor inhibitory activity (4).

Most of the *Klebsiella* strains were resistant to piperacillin-tazobactam and all of the cephalosporins, but once again, avibactam protected ceftazidime against the majority of isolates, bringing the MIC_{90} of ceftazidime down from >128 to 4 μg/ml.

The class A ESBLs of the TEM and SHV groups all occurred among the *E. coli* and *Klebsiella* strains. For these ESBL enzymes, potentiation of ceftazidime by avibactam was generally ≥256-fold, while the non-ESBL members of these two enzyme groups showed lower ceftazidime MICs and, consequently, a more modest (≤16-fold) potentiation with avibactam. For the strains expressing cefotaximases (CTX-M enzymes), levels of potentiation of ceftazidime by avibactam were 16- to >128-fold. Potentiation by avibactam was observed for both ceftazidime-resistant (ceftazidime MIC, ≥8 μg/ml) and -susceptible (ceftazidime MIC, ≤4 μg/ml) isolates.

KPC β-lactamases were found as the sole enzymes in seven *K. pneumoniae*, one *E. coli*, and one *E. cloacae* strain. Against these enzymes, avibactam usually potentiated the activity of ceftazidime ≥128-fold, with MICs reduced from 64 to >128 μg/ml to 0.25 to 8 μg/ml. One strain of *K. pneumoniae* producing a KPC-3 enzyme had an MIC of 8 μg/ml for the ceftazidime-avibactam combination. No other β-lactamases were detected in this isolate, and it is possible that the elevated MIC was due in part to an increased permeability barrier.

A few isolates contained other, nonclassical class A β-lactamases. Good synergy was seen against a PER-1-producing strain of *E. coli*, where the MIC of ceftazidime was reduced from >128 μg/ml to 1 μg/ml. Against other enzymes, such as GES-2, PSE-1, and PSE-4, the levels of potentiation of ceftazidime by avibactam were 4-, 32-, and 4-fold, respectively (see Table S1 in the supplemental material).

Against two *E. coli* isolates which contained only a class D OXA-1 enzyme, avibactam potentiated the activity of ceftazidime

TABLE 2 *In vitro* activities of ceftazidime, ceftazidime-avibactam, and comparators against clinical isolates of *Enterobacteriaceae*

Organism(s) (no. of isolates) and drug(s)	MIC (μg/ml)			
	Range	MIC_{50}	MIC_{90}	% S[a]
All *Enterobacteriaceae* (169)				
Ceftazidime	0.25–>128	16	>128	49
Ceftazidime-avibactam[b]	≤0.12–128	0.25	2	99
Cefotaxime	≤0.12–>128	8	>128	54
Ceftriaxone	≤0.12–>128	16	>128	50
Cefepime	≤0.12–128	0.5	128	80
Piperacillin-tazobactam[c]	≤0.12–>128	8	>128	64
Imipenem	≤0.12–128	0.25	2	95
Escherichia coli (46)				
Ceftazidime	0.25–>128	16	>128	46
Ceftazidime-avibactam[b]	≤0.12–128	0.25	1	98
Cefotaxime	≤0.12–>128	4	128	67
Ceftriaxone	≤0.12–>128	4	128	61
Cefepime	≤0.12–>128	1	32	85
Piperacillin-tazobactam[c]	≤0.12–>128	4	>128	78
Imipenem	≤0.12–16	0.25	0.5	98
Klebsiella spp. (46)				
Ceftazidime	0.25–>128	>128	>128	28
Ceftazidime-avibactam[b]	≤0.12–8	1	4	100
Cefotaxime	≤0.12–>128	32	>128	30
Ceftriaxone	≤0.12–>128	64	>128	22
Cefepime	≤0.12–>128	8	128	59
Piperacillin-tazobactam[c]	≤0.12–>128	>128	>128	35
Imipenem	≤0.12–128	0.25	16	87
Citrobacter freundii (15)				
Ceftazidime	0.5–>128	16	128	40
Ceftazidime-avibactam[b]	≤0.12–0.5	0.25	0.5	100
Cefotaxime	0.25–64	8	64	53
Ceftriaxone	0.25–128	8	64	60
Cefepime	≤0.12–1	≤0.12	0.5	100
Piperacillin-tazobactam[c]	0.5–128	2	64	80
Imipenem	≤0.12–1	0.25	0.5	100
Enterobacter spp. (16)				
Ceftazidime	0.25–>128	128	>128	31
Ceftazidime-avibactam[b]	≤0.12–64	1	8	94
Cefotaxime	≤0.12–>128	64	>128	31
Ceftriaxone	0.25–>128	64	>128	25
Cefepime	≤0.12–>128	2	>128	63
Piperacillin-tazobactam[c]	0.5–>128	128	>128	38
Imipenem	≤0.12–64	0.5	32	88
Morganella morganii (11)				
Ceftazidime	0.25–>128	1	64	64
Ceftazidime-avibactam[b]	≤0.12–0.25	≤0.12	0.25	100
Cefotaxime	≤0.12–32	0.5	32	64
Ceftriaxone	≤0.12–16	0.25	8	91
Cefepime	≤0.12–0.25	≤0.12	≤0.12	100
Piperacillin-tazobactam[c]	≤0.12–64	0. 5	16	91
Imipenem	1–2	1	2	100
Providencia spp. (19)				
Ceftazidime	0.25–16	2	8	95
Ceftazidime-avibactam[b]	≤0.12–1	0.5	1	100
Cefotaxime	≤0.12–8	1	2	100
Ceftriaxone	≤0.12–4	≤0.12	1	100
Cefepime	≤0.12–4	0.5	2	100
Piperacillin-tazobactam[c]	0.25–>128	8	>128	79
Imipenem	0.5–4	1	4	100
Proteus spp. (13)				
Ceftazidime	0.25–>128	0.5	32	85
Ceftazidime-avibactam[b]	≤0.12–1	≤0.12	1	100
Cefotaxime	≤0.12–>128	8	>128	54
Ceftriaxone	≤0.12–>128	>128	>128	31
Cefepime	≤0.12–>128	0.5	1	92
Piperacillin-tazobactam[c]	≤0.12–8	1	4	100
Imipenem	1–4	2	4	100
Serratia marcescens (3)				
Ceftazidime	4–32			
Ceftazidime-avibactam[b]	0.25–0.5			
Cefotaxime	32–256			
Ceftriaxone	32–256			
Cefepime	2–64			
Piperacillin-tazobactam[c]	1–32			
Imipenem	0.25–0.5			

[a] % S, percentage of susceptible isolates according to CLSI breakpoint criteria for *Enterobacteriaceae* (8).
[b] Since there is not yet an approved breakpoint for ceftazidime-avibactam, the values reported here are for 8 μg/ml ceftazidime in the presence of a fixed concentration of 4 μg/ml avibactam.
[c] Values reported are for the piperacillin MIC in the presence of a fixed concentration of 4 μg/ml tazobactam.

≥4-fold, with MICs reduced from 0.5 and 1 μg/ml to ≤0.12 and 0.25 μg/ml. This weak potentiation is in agreement with the work of Poirel et al. (9), who stated that broad-spectrum cephalosporins are "slightly hydrolyzed" by OXA-1.

All 15 *C. freundii* isolates contained just a chromosomal class C β-lactamase and were susceptible to ceftazidime-avibactam, cefepime, and imipenem.

Five strains of ceftazidime-sensitive *E. cloacae* showed 2- to 8-fold improvements in the ceftazidime MIC in the presence of avibactam. Improvements of >64-fold were observed against 10/11 ceftazidime-resistant strains (including 2 strains which also contained KPC enzymes). Barnaud et al. (10) reported on two strains of *Enterobacter* containing AmpC variants resistant to cefepime (FEP^r) as well as ceftazidime. One FEP^r *E. cloacae* isolate,

TABLE 3 Summary of *in vitro* potentiation of ceftazidime by avibactam against different enzyme types in isolates with ceftazidime MICs of >8 μg/ml

Enzyme class	Subclass	*n*	Fold reduction in MIC	
			Range	Median
A[a]	TEM ESBL	9	64–≥512	≥256
	SHV ESBL	6	64–≥512	≥256
	CTX-M	6	16–≥128	64
	KPC	9	32–≥512	≥256
C[b]		26	4–≥512	≥128
Multienzyme producers[c]		18	2–≥512	≥128

[a] Isolates contained a single class A enzyme.
[b] Isolates contained a single class C enzyme. Four isolates also contained a TEM-1 enzyme.
[c] Seventeen of eighteen isolates contained ≥2 class A enzymes, 5/18 isolates also contained a class C enzyme, and 3/18 isolates also contained a class D enzyme.

containing a six-amino-acid deletion in the H-10 helix (10), gave poor potentiation of ceftazidime by avibactam, with the ceftazidime MIC reduced from >128 to 64 μg/ml. A FEP^r strain of *Enterobacter aerogenes* containing an L-293-P mutation (11) showed better potentiation, with the MIC of ceftazidime falling from >128 to 4 μg/ml in the presence of 4 μg/ml avibactam.

Ceftazidime is a poor substrate for most of the β-lactamases produced by the *Proteus*/*Providencia*/*Morganella* group of organisms. Consequently, clinical isolates from these genera are typically ceftazidime sensitive, although synergy with avibactam may still be seen.

Two of 6 *Providencia rettgeri* strains were ceftazidime resistant and showed a 32-fold reduction in ceftazidime MIC in the presence of avibactam. The remaining 4 isolates were ceftazidime sensitive, but potentiation was still seen with avibactam.

The 13 *Providencia stuartii* isolates had ceftazidime MICs of 0.5 to 4 μg/ml and generally showed 2- to 8-fold reductions in MIC in the presence of avibactam.

Two of 4 *Proteus mirabilis* isolates were sensitive to ceftazidime alone, with MICs of 0.25 μg/ml. Against the two remaining strains, the presence of avibactam reduced the ceftazidime MIC >128-fold, from 32 and >128 μg/ml to ≤0.12 and 1 μg/ml, respectively.

The 9 *Proteus vulgaris* isolates, with either inducible or stably derepressed expression of the chromosomal class A enzyme CumA, were all susceptible to ceftazidime (MICs of ≤1 μg/ml), but once again, ceftazidime was potentiated >4-fold by avibactam against many of these isolates.

To conclude, avibactam potentiated ceftazidime activity against the majority of ceftazidime-resistant clinical isolates of the family *Enterobacteriaceae*, rendering the vast majority of isolates susceptible to ceftazidime. Ceftazidime MICs were also improved for a number of ceftazidime-sensitive isolates when ceftazidime was combined with avibactam. These data are in broad agreement with those recently reported by Sader et al. (12). Potentiation was more marked with fixed concentrations of avibactam than with fixed ratios of ceftazidime-avibactam.

The strain collection used in this study was enriched with isolates producing broad-spectrum β-lactamases, mostly class A enzymes. Thus, the MIC_{50}/MIC_{90} ratios of comparator agents may be higher than expected. The only other β-lactamase inhibitor used in this study was tazobactam, in combination with piperacillin. Other marketed β-lactam–β-lactamase inhibitor combinations were not studied, but we would expect them to be no more active than piperacillin-tazobactam and, in the majority of cases, less active.

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