Colistin Does Not Potentiate Ceftazidime-Avibactam Killing of Carbapenem-Resistant *Enterobacteriaceae* *In Vitro* or Suppress Emergence of Ceftazidime-Avibactam Resistance

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ABSTRACT We tested ceftazidime-avibactam and colistin against 24 carbapenem-resistant *Enterobacteriaceae* (CRE) isolates by time-kill studies. Ceftazidime-avibactam at 0.25×, 1×, and 4× the MIC was bactericidal against 8%, 21%, and 88% of the isolates, respectively. Colistin (2 µg/ml) was bactericidal against 83% (12 h) and 42% (24 h) of the isolates. In combination, synergy and antagonism were identified against 13% and 46% of the isolates, respectively. The combination did not suppress ceftazidime-avibactam resistance. Colistin plus ceftazidime-avibactam did not provide a benefit over ceftazidime-avibactam against most CRE isolates.

KEYWORDS ceftazidime-avibactam, colistin, *Klebsiella pneumoniae*, CRE, synergy, antagonism, *Klebsiella*

Ceftazidime-avibactam is a novel β -lactam- β -lactamase inhibitor with *in vitro* activity against carbapenem-resistant *Enterobacteriaceae* (CRE) isolates expressing *Klebsiella pneumoniae* carbapenemases (KPCs) and OXA-48 but not other Ambler class D or class B β -lactamases. Ceftazidime-avibactam MICs are higher against KPC-3-producing *K. pneumoniae* isolates than against KPC-2-producers (1). We previously showed a stepwise increase in ceftazidime-avibactam MICs against KPC-2-producing *K. pneumoniae* isolates that harbored extended-spectrum β -lactamases (ESBLs) and mutations in the *ompK36* porin gene (1). We postulated that the entry of ceftazidime-avibactam into the periplasmic space may be at least partially dependent upon outer membrane permeability, a contention that is supported by the characterization of ceftazidime-avibactam-resistant *Pseudomonas aeruginosa* isolates (2).

Emerging clinical data demonstrate that ceftazidime-avibactam is well tolerated among patients with CRE infection, and treatment with the agent achieves clinical response rates that are superior to those attained with regimens that include colistin or an aminoglycoside (3–6). At our center, however, ceftazidime-avibactam resistance has emerged in ~10% of the CRE-infected patients treated with the drug. It is unclear if combining ceftazidime-avibactam with another CRE-active agent would improve treatment effectiveness and limit the emergence of resistance. In prior studies, we identified *in vitro* synergy with colistin-carbapenem combinations against extensively drug-resistant *Acinetobacter baumannii* and carbapenem-resistant *K. pneumoniae* (CRKp) isolates (7–10). The interaction between agents is consistent with a model in which membrane permeabilization by colistin facilitates increased access of the carbapenem to its target sites. We hypothesized that colistin likewise would potentiate the *in vitro* killing of CRE by ceftazidime-avibactam and suppress the emergence of ceftazidime-avibactam resistance.

To test our hypotheses, we studied 24 CRE isolates from unique patients, including 16 CRKp isolates and 8 CRE isolates of other species. β -Lactamase profiles and porin

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FIG 1 Mean log kills of all 24 CRE isolates at ceftazidime-avibactam concentrations ranging from 0.25 to 4× the MIC. Avibactam was fixed at 4 μ g/ml in all experiments. The lower limit of detection was 1.7 log CFU/ml, which is represented by the dashed line. Error bars show the standard deviation at each time point.

gene sequences of CRKp isolates were established, as described previously (10–13). We included CRKp isolates harboring KPC-2 and KPC-3, as well as those with and without *ompK36* porin gene mutations. Among other CRE isolates, the presence of *bla*_{CTX-M}, *bla*_{KPC}, *bla*_{SHV}, and *bla*_{TEM} was assessed by PCR. Isolates were stored at -80°C and subcultured twice on Mueller-Hinton agar prior to testing. The MICs of colistin, ceftazidime, and ceftazidime-avibactam were determined using Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution methods with cation-adjusted Mueller-Hinton broth (14). Avibactam was tested at a fixed concentration of 4 μ g/ml.

Next, we performed time-kill assays using a 1×10^6 CFU/ml inoculum in 10 ml of cation-adjusted Mueller-Hinton broth at 37°C . Isolates were tested in the presence of ceftazidime-avibactam at 0.25×, 1×, and 4× the MIC (avibactam was fixed at 4 μ g/ml), 2 μ g/ml colistin, and a combinations of colistin (2 μ g/ml) with each ceftazidime-avibactam concentration. Surviving bacteria were enumerated at 0, 4, 8, 12, and 24 h; the lower limit of detection was 50 CFU/ml (1.7 log₁₀ CFU/ml). Log kills were calculated as the log₁₀ CFU per milliliter at a time point of interest minus the log₁₀ CFU per milliliter at time zero. Bactericidal responses were defined as a ≥ 3 -log kill from the starting inoculum. Regrowth was characterized by bactericidal activity at 12 h that was no longer present at 24 h due to an increased bacterial burden. To assess the interaction of two-drug combinations, we defined synergy as a ≥ 2 -log greater kill in combination compared to that of the most active single agent. Antagonism was considered ≥ 1 -log lower kill in combination compared to that of the most active single agent. All other interactions were considered indifferent.

Ninety-two percent (22/24) of the isolates harbored *bla*_{KPC}, and 42% (10/24) and 50% (12/24) of the isolates carried KPC-3 and KPC-2, respectively. Other β -lactamases were common, including TEM (96% [23/24]), SHV (83% [20/24]), and CTX-M (29% [7/24]) variants. The median ceftazidime, ceftazidime-avibactam, and colistin MICs were 512 (range, 64 to 2,048 μ g/ml), 1 (range, 0.25 to 8 μ g/ml), and 0.25 μ g/ml (range, 0.125 to 0.5 μ g/ml), respectively. As a single agent, the mean log kills with ceftazidime-avibactam at 0.25×, 1×, and 4× the MIC were 2.65 (range, -3.48 to 3.69), -1.12 (range, -4.88 to 2.62), and -3.89 (range, -6.24 to 1.97) CFU/ml at 12 h (Fig. 1). The corresponding bactericidal rates were 8% (2/24), 21% (5/24), and 88% (21/24), respectively. Regrowth was identified in 24% (5/21) of the isolates exposed to ceftazidime-avibactam at 4× the MIC. Mean 24-h log kills were 2.94 (range, 1.42 to 4.49), -1.45 (range, -6.19 to 4.38), and -3.14 (range, -6.25 to 4.17), respectively (Fig. 1). There were no differences in ceftazidime-avibactam mean log kills against CRKp by KPC subtype or porin genotype (Table 1). Colistin was bactericidal against 88% (21/24) and 42% (10/24) of the isolates at 12 and 24 h, respectively (Table 1). The mean log kills were greater at 12 h (-5.08 CFU/ml) than at 24 h (-1.38 CFU/ml; $P = 0.0001$); regrowth between 12 and 24 h occurred in 83% (20/24) of isolates.

Combinations of colistin plus ceftazidime-avibactam at 0.25×, 1×, or 4× the MIC

TABLE 1 Molecular characteristics, drug MICs, and time-kill results of 24 CRE isolates

Isolate	<i>bla</i> _{KPC} WT or variant (<i>ompK36</i> genotype) ^a	MIC (μg/ml) ^b		Log kill at 12 h (CFU/ml) ^c				Log kill at 24 h (CFU/ml) ^c				Interpretation at 12 h		Interpretation at 24 h	
		C-A	COL	C-A at 4 × the MIC	2 μg/ml COL	C-A plus COL	Interpretation at 12 h	C-A at 4 × the MIC	2 μg/ml COL	C-A plus COL	Interpretation at 24 h		Interpretation at 12 h		Interpretation at 24 h
Kp-347	KPC-2 (WT)	0.5	0.25	−0.57	−3.70	−5.96	Synergy	−1.28	−0.99	−5.96	Synergy		Synergy	−5.96	
Kp-142	KPC-2 (WT)	0.5	0.25	1.97	−6.11	−6.10	Indifferent	4.17	−0.01	−0.62	Indifferent		Indifferent	−0.62	
Kp-465	KPC-2 (WT)	1	0.5	−6.19	−2.01	−6.26	Indifferent	−6.19	3.73	−0.85	Antagonism		Antagonism	−0.85	
Kp-669	KPC-2 (AA134-135)	0.5	0.25	−4.65	−6.18	−6.15	Indifferent	−6.26	−2.67	−6.15	Indifferent		Indifferent	−6.15	
Kp-115	KPC-2 (AA134-135)	1	0.125	−3.80	−3.34	−6.25	Synergy	−4.95	2.54	−0.33	Antagonism		Antagonism	−0.33	
Kp-436	KPC-2 (AA134-135)	1	0.25	−4.47	−6.24	−6.25	Indifferent	−6.25	−2.22	−2.18	Antagonism		Antagonism	−2.18	
Kp-155	KPC-2 (AA134-135)	2	0.25	−3.46	−6.23	−6.04	Indifferent	2.29	−3.85	−2.13	Antagonism		Antagonism	−2.13	
Kp-873	KPC-2 (IS5)	1	0.25	−3.24	−6.10	−6.24	Indifferent	−4.07	−0.98	−0.44	Antagonism		Antagonism	−0.44	
Kp-937	KPC-2 (IS5)	2	0.25	−3.67	−6.19	−6.21	Indifferent	−4.35	1.39	−6.21	Indifferent		Indifferent	−6.21	
Kp-306	KPC-3 (WT)	0.5	0.5	2.15	−4.18	−6.15	Indifferent	3.33	3.44	−6.15	Synergy		Synergy	−6.15	
Kp-779	KPC-3 (WT)	1	0.25	−4.80	−6.23	−6.26	Indifferent	−2.39	−6.23	−6.26	Indifferent		Indifferent	−6.26	
Kp-929	KPC-3 (WT)	1	0.125	−4.79	−6.04	−6.15	Indifferent	−4.79	−2.66	−2.90	Antagonism		Antagonism	−2.90	
Kp-928	KPC-3 (WT)	2	0.5	−3.33	1.93	−3.85	Indifferent	−3.99	4.15	−1.70	Antagonism		Antagonism	−1.70	
Kp-930	KPC-3 (WT)	4	0.06	−4.53	−6.24	−6.14	Indifferent	−6.13	−3.82	−6.14	Indifferent		Indifferent	−6.14	
Kp-913	KPC-3 (IS5)	0.5	0.25	−3.19	−6.02	−6.05	Indifferent	2.68	−4.11	−1.70	Antagonism		Antagonism	−1.70	
Kp-380	No KPC (IS5)	4	0.5	−3.99	−6.13	−6.15	Indifferent	−0.71	1.74	−2.81	Synergy		Synergy	−2.81	
En-102	KPC-2	0.5	0.06	−3.75	−6.07	−6.11	Indifferent	−4.45	−6.07	−6.11	Indifferent		Indifferent	−6.11	
En-24	KPC-2	1	0.06	−3.47	−6.02	−6.03	Indifferent	−4.32	−3.28	−6.03	Indifferent		Indifferent	−6.03	
En-150	KPC-3	2	0.125	−4.26	−6.09	−6.12	Indifferent	−4.86	−6.09	−3.92	Antagonism		Antagonism	−3.92	
En-6	KPC-3	4	0.125	−3.83	−6.09	−6.09	Indifferent	−4.35	2.96	−6.09	Indifferent		Indifferent	−6.09	
Ec-13	No KPC	0.25	0.125	−3.96	−0.23	−6.18	Synergy	−4.61	3.72	−2.07	Antagonism		Antagonism	−2.07	
Ec-42	KPC-2	1	0.06	−6.19	−6.23	−6.21	Indifferent	−1.61	−6.23	−6.21	Indifferent		Indifferent	−6.21	
Kl-1173	KPC-3	4	0.06	−4.83	−6.17	−6.09	Indifferent	−6.13	−4.39	−6.09	Indifferent		Indifferent	−6.09	
Ct-83	KPC-3	8	0.06	−6.25	−6.25	−6.28	Indifferent	−6.25	−3.10	−2.73	Antagonism		Antagonism	−2.73	

^aWT, wild type; *ompK36* genotypes included wild type (WT), a 6-bp insertion that encodes glycine and aspartic acid at amino acids 134 and 135 (AA134-135), and an ISS insertion sequence, as previously reported (6).^bC-A, ceftazidime-avibactam; COL, colistin.^cTime-kill experiments were performed at least twice; representative results are provided.

FIG 2 Mean log kills of all 24 CRE isolates by ceftazidime-avibactam (C-A, 4× the MIC), 2 μg/ml colistin alone, or a combination of both agents. The lower limit of detection was 1.7 log CFU/ml, which is represented by the dashed line. Error bars show the standard deviation at each time point.

were bactericidal against all isolates at 12 h (Fig. 2). The corresponding combinations met the definition of *in vitro* synergy at 12 h against 17% (4/24), 13% (3/24), and 13% (3/24) of the isolates, respectively. Regrowth occurred in 83% (20/24), 71% (17/24), and 54% (13/24) of the isolates, respectively. *In vitro* antagonism between colistin and ceftazidime-avibactam at 4× the MIC was identified in 46% (11/24) of the isolates. Among isolates demonstrating antagonistic responses, regrowth occurred in combination, but not in the presence of single agents (ceftazidime-avibactam [$n = 8$], colistin [$n = 2$] or both [$n = 1$]) (Table 1). The rates of *in vitro* bactericidal, synergistic, and antagonistic activities did not vary by species, KPC subtype, or porin genotype.

To evaluate the emergence of drug resistance, we repeated time-kill analyses on 4 CRKp isolates exhibiting antagonism. Exposure to the combination of ceftazidime-avibactam (4× the MIC) and colistin (2 μg/ml) resulted in bactericidal killing of each isolate below the limit of detection at 12 h, followed by regrowth at 24 h (mean regrowth to 5.55 log₁₀ CFU/ml). Antagonism between ceftazidime-avibactam and colistin at 24 h was evident against 75% (3/4) of the isolates in repeat experiments. The criteria for antagonism were not met for one isolate due to bacterial regrowth in the presence of each drug individually and in combination. At 24 h following exposure to the combination, 5 colonies of the 4 CRKp isolates were collected for ceftazidime-avibactam and colistin MIC determinations. Across all replicates, colistin MICs did not change from baseline (data not shown). On the other hand, ceftazidime-avibactam resistance emerged in at least one replicate of each isolate (Table 2).

Ceftazidime-avibactam is a major advance in the treatment of CRE infections, but recent reports have described the emergence of resistance among KPC-producing *K. pneumoniae* isolates (3, 4, 15–17). As such, it is important to determine if combination regimens can suppress ceftazidime-avibactam resistance amplification. Colistin is a logical partner agent with ceftazidime-avibactam given its retained *in vitro* activity against CRE worldwide (18) and documented synergy with other β-lactam agents (8, 9). Our present analysis, however, suggests that colistin does not potentiate the *in vitro*

TABLE 2 Ceftazidime-avibactam MICs against *K. pneumoniae* isolates following exposure to ceftazidime-avibactam and colistin

Replicate isolate ^a	Ceftazidime-avibactam MIC (μg/ml)			
	Kp-115	Kp-436	Kp-465	Kp-873
Baseline	1	1	1	1
A	1	64	32	1
B	1	64	1	1
C	32	64	1	32
D	2	64	2	1
E	1	2	2	1

^aReplicate isolates A to E were selected from each parent isolate after 24 h of exposure to the combination of ceftazidime-avibactam and colistin.

killing of most CRE isolates by ceftazidime-avibactam, nor does it suppress ceftazidime-avibactam resistance. In fact, the emergence of resistance may be underestimated in this study since resistant subpopulations can exist below the limit of detection for standard time-kill analysis. Unlike mitigated responses among colistin-carbapenem combinations (9), the attenuated killing activity of colistin plus ceftazidime-avibactam was not predicted by *ompK36* porin genotypes. While more definitive studies are needed to confirm our findings, the data presented here are sufficient to justify exploring alternative ceftazidime-avibactam combination regimens. We cannot recommend the use of colistin among patients receiving ceftazidime-avibactam until more conclusive data are available.

The *in vitro* killing activity of colistin reported here is consistent with our previous time-kill analyses (9, 10). The cumulative data indicate that *in vitro* exposure of most CRE isolates to clinically relevant concentrations of colistin results in initial bactericidal responses, followed by exponential regrowth. We also demonstrated that ceftazidime-avibactam was bactericidal at 4× the MIC against CRE, but regrowth occurred for a minority of isolates. These data corroborate a prior study evaluating the *in vitro* activity of ceftazidime-avibactam at 2×, 4×, and 8× the MIC against genetically diverse *Enterobacteriaceae* (19). In the earlier study, supplementing time-kill assays with additional avibactam at 6 h suppressed the regrowth of certain isolates. It is unclear if avibactam is dissipated during fixed-concentration testing or if concentrations of >4 µg/ml are required to suppress bacterial growth over a 24-h period (20). The mechanisms for regrowth in the presence of colistin or ceftazidime-avibactam are unknown and will be the focus of future study. One hypothesis is that colistin regrowth is mediated by PmrAB or PhoPQ two-component regulatory systems, which have been implicated in modifications of cell membrane lipopolysaccharides (LPS) that reduce the affinity for colistin (21, 22). A similar two-component regulatory system in *P. aeruginosa*, ParRS, also mediates adaptive responses to colistin, including LPS modifications, drug efflux, and reduced outer membrane permeability (23). Likewise, it is possible that ceftazidime-avibactam regrowth is associated with mutations in genes encoding carbapenemases or porins (24).

Promising candidate antibiotics to combine with ceftazidime-avibactam in future studies include carbapenems, gentamicin, and fosfomycin. In both *in vitro* passage and clinical isolates, ceftazidime-avibactam resistance is mediated by Ω-loop mutations in plasmid-borne *bla*_{KPC-3} (15, 25), which restore carbapenem susceptibility in most isolates. Carbapenem and ceftazidime-avibactam combinations may mitigate the emergence of *bla*_{KPC} Ω-loop mutations or, alternatively, treat resistant subpopulations; however, selection for other carbapenem resistance mechanisms, like *ompK36* mutations, may occur (24). Gentamicin is rapidly bactericidal against CRE *in vitro* and not affected by *ompK36* mutations (10) but it is limited by nephrotoxicity and suboptimal pharmacokinetics in the clinic (26). Fosfomycin demonstrates synergy with ceftazidime-avibactam against *P. aeruginosa* (2), but clinical experience with systemic formulations against CRE infections is limited, and resistance may emerge during treatment (27). At present, the optimal ceftazidime-avibactam combination regimen remains unknown.

In conclusion, we have demonstrated that colistin does not reliably potentiate the *in vitro* killing activity of ceftazidime-avibactam in static time-kill models. Until further data are available, positive interactions between ceftazidime-avibactam and colistin should not be assumed. Antagonistic interactions against some CRE isolates may be dependent upon the genetics of strains and testing conditions, as also suggested by a recent study in which antagonism was demonstrated against one of four CRE isolates (28). In follow-up studies, we will investigate and model colistin and ceftazidime-avibactam interactions in dynamic models *in vitro* and test other agents in combination with ceftazidime-avibactam over prolonged treatment courses. It will also be important to compare the activities of ceftazidime-avibactam against CRE to that of β-lactam-β-lactamase inhibitors currently in development.

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