Activity of ceftazidime-avibactam alone or in combination with polymyxin B against carbapenem-resistant *Klebsiella pneumoniae* in a tandem *in vitro* time kill analysis*in vivo Galleria mellonella* survival model

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Introduction

- The optimal antimicrobial therapy for infections caused by carbapenem-resistant *Klebsiella pneumoniae* (CRKP) remains unknown
- Ceftazidime-avibactam (CAZ-AVI) has emerged as the treatment of choice for KPC infections due to its *in vitro* activity, low toxicity, and superiority over traditional treatment regimens
- Although CAZ-AVI is often used in combination clinically, data to support this practice is limited and there is currently a paucity of literature on the efficacy of CAZ-AVI in combination with polymyxin B (PB)
- Demonstrating synergy between CAZ-AVI and PB *in vitro* will help elucidate the rate and extent of bactericidal activity
- Mammalian systems are time-consuming, expensive and require full ethical considerations while pre-mammalian infection models, like *Galleria mellonella*, provide preliminary *in vivo* data quickly and inexpensively
- Utilizing a pre-mammalian *in vivo* model with an immune system and host response can help to validate the results seen in the *in vitro* analyses and determine if this combination warrants investigation in more cumbersome animal models or in humans

Objectives

- The first object of this study was to evaluate the activity of CAZ-AVI and PB alone and in combination against KPC-producing *Klebsiella pneumoniae* in *in vitro* time kill experiments
- The second objective of this study was to verify *in vitro* results by evaluating the efficacy of CAZ-AVI and PB alone and in combination against KPC-producing *Klebsiella pneumoniae* in an *in vivo Galleria mellonella* survival model

Methods

- 3 clinical KPC-producing *K. pneumoniae* strains used for all experiments
  - *Klebsiella pneumoniae* ATCC 700603 QC strain
- MICs and time kill analyses performed in triplicate according to CLSI guidelines
  - Samples at 0, 2, 4, 6, and 24 hours
- Individual drugs tested at ¼, ½, 1, 2 and 4 x MIC
- Combinations tested at highest concentration of each drug alone which showed the least *in vitro* activity
- Bactericidal: ≥3 log10 CFU/mL reduction in bacterial density compared to the starting inoculum
- Synergy: ≥2 log10 CFU/mL decrease at 24 hours with the combination compared to the most active single agent alone
- Antagonism: ≥2 log10 CFU/mL increase at 24 hours with the combination compared to the most active agent alone.
- Indifference: 0-<2log10 CFU/mL change at 24 hours with the combination compared to the most active single agent alone
- *G. mellonella* at final instar stage acquired from wholesaler and used within 7 days
- Groups of 10 healthy larvae weighing 250-350 mg and free of any gray markings used for each experiment
- Each experiment included two control groups of larvae: a group injected with PBS once or twice and another untouched group
- Larvae were inoculated with a predetermined lethal *K. pneumoniae* inoculum followed by the test drug(s) within 1 h after inoculation
- Antibiotic concentrations used in vivo were based on observations from the *in vitro* time-kill analyses
- After injection, larvae were incubated at 37°C with survival measured daily by manual stimulation for 5 days
- Larvae survival was plotted via Kaplan-Meier method and survival differences between groups was compared via log rank test with Bonferroni correction for multiple comparisons

Results

Table 1. Characteristics of KPC-producing *K. pneumoniae* strains

Isolate	CAZ-AVI MIC (mg/L)	Interpretive Category^	PB MIC (mg/L)	Interpretive Category^	Resistance mechanism*	Ompk35 Mutation	Ompk36 Mutation
KPC1	1	S	0.25	S	KPC-3	Yes	No
KPC2	8	S	0.25	S	KPC-3	Yes	Yes
KPC3	16	R	64	R	KPC-3	Yes	No

S- susceptible, R – resistant

*None of the isolates harbored Ω-loop mutations

^Interpretation based on Clinical and Laboratory Standards Institute M100-S27

Figure 1 - Time kill analyses for ceftazidime-avibactam and polymyxin B monotherapy

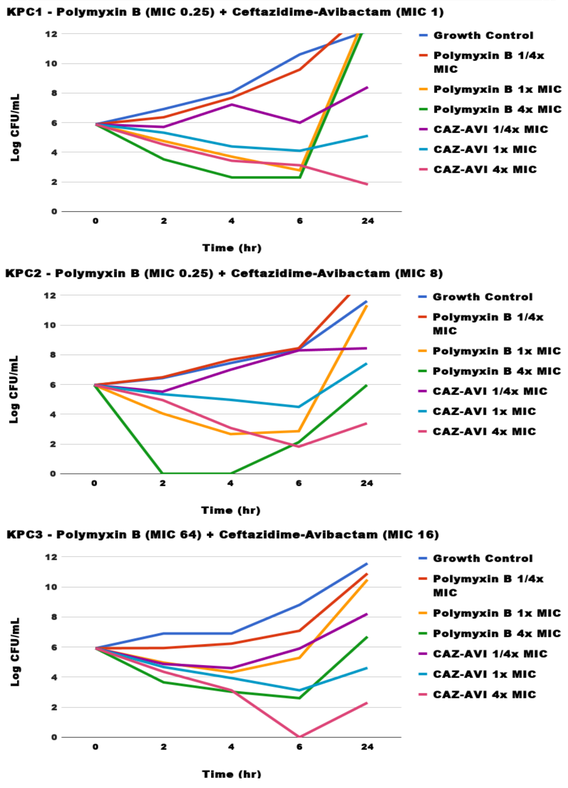


Table 2. Time kill results for CAZ-AVI monotherapy, PB monotherapy, and combination therapy

Isolate	CAZ-AVI monotherapy log-change at 24h (CFU/mL)						PB monotherapy log-change at 24h (CFU/mL)						Combination therapy log-change at 24h (CFU/mL)		Interpretation
	GC	¼x MIC	½x MIC	1x MIC	2x MIC	4x MIC	GC	¼x MIC	½x MIC	1x MIC	2x MIC	4x MIC	¼x MIC	½x MIC	
KPC1 (MIC C-A 1, PB 0.25)	+6.31	+2.52	-3.41	-3.10	-2.76	-4.07	+6.31	+7.77	+7.74	+7.71	+7.04	+7.15	+3.17	-	Indifference (+0.66)
KPC2 (MIC C-A 8, PB 0.25)	+5.65	+2.47	+2.25	+1.46	-0.25	-2.57	+5.65	+7.35	+5.26	+5.39	+5.18	+0.00	-	+2.96	Indifference (+0.71)
KPC3 (MIC C-A 16, PB 64)	+5.65	+2.29	-3.14	-1.30	-5.92	-3.62	+5.65	+4.97	+5.25	+4.57	+4.45	+0.77	+3.58	-	Indifference (+1.29)

CFU – colony forming units, CAZ-AVI – ceftazidime-avibactam, GC – growth control, MIC – minimum inhibitory concentration, PB – polymyxin B

Figure 2 - Time kill analyses for ceftazidime-avibactam, polymyxin B, and in combination

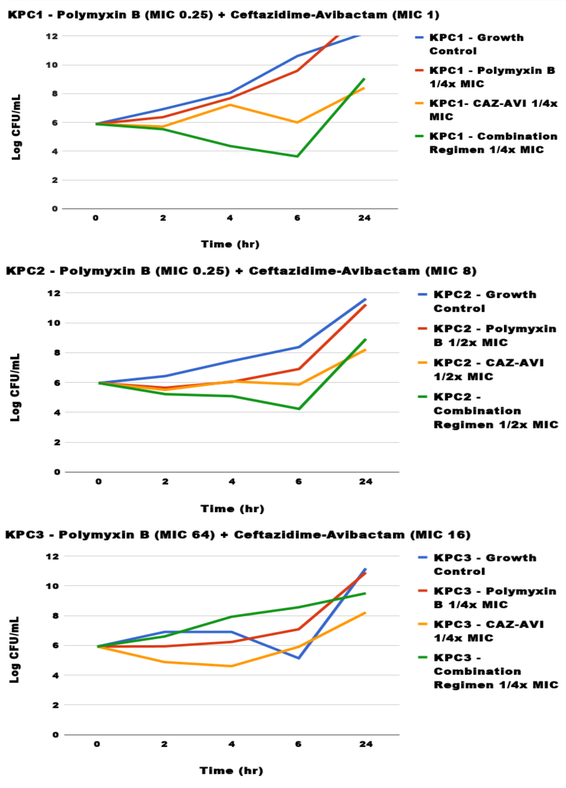


Figure 3. Healthy (left) and infected (right) *G. mellonella* larvae

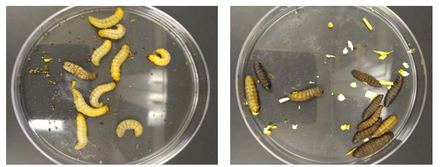
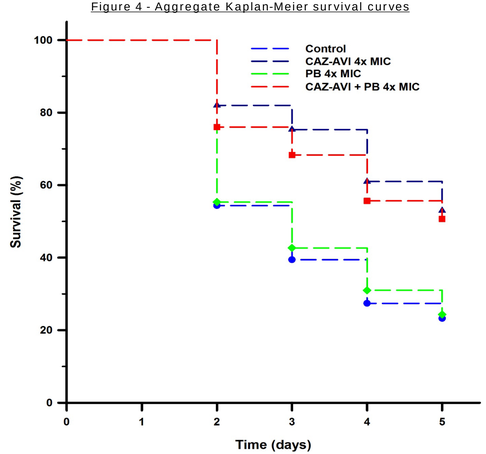


Table 3. Aggregate log-rank pairwise comparison of combination and monotherapy*

	Untreated Controls	
	X^2	*P* value
Ceftazidime-avibactam	66.420	<0.001
Polymyxin B	0.072	0.788
Ceftazidime-avibactam + Polymyxin B	50.420	<0.001
	CAZ-AVI at 4x MIC	
Ceftazidime-avibactam + Polymyxin B	1.186	0.276

* Each antibiotic at 4x MIC compared to untreated controls, CAZ-AVI monotherapy, and adjusted for isolate

Figure 4 - Aggregate Kaplan-Meier survival curves



Conclusions

- CAZ-AVI monotherapy at 4x MIC was bactericidal against 2/3 KPC isolates
- PB monotherapy, regardless of concentration, was not bactericidal at 24 hours against any isolate
- Neither agent alone was bactericidal at 1/4x or 1/2x MIC
- When combined with PB at 1/4x or 1/2x MIC, CAZ-AVI was not synergistic or bactericidal regardless of isolate
- In the *G. mellonella* survival model, the combination of CAZ-AVI and PB did not show a survival advantage over CAZ-AVI monotherapy
- These results indicate that there does not seem to be reliably favorable interactions between CAZ-AVI and PB
- Future *in vitrolin vivo* studies including more isolates and clinical data in humans are needed to confirm these findings

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