J Antimicrob Chemother 2018; **73**: 2405–2410
doi:10.1093/jac/dky213 Advance Access publication 22 June 2018

Journal of Antimicrobial Chemotherapy

Ceftazidime/avibactam versus standard-of-care agents against carbapenem-resistant Enterobacteriaceae harbouring *bla*$_{KPC}$ in a one-compartment pharmacokinetic/pharmacodynamic model

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Received 8 February 2018; returned 24 March 2018; revised 4 May 2018; accepted 11 May 2018

Background: 'Last-line' antimicrobial usage has promoted the emergence of MDR bacteria. Production of *Klebsiella pneumoniae* carbapenemases (KPCs) is increasingly common and leads to resistance to most antimicrobials. However, ceftazidime/avibactam demonstrates activity against KPC-producing strains. Ceftazidime/avibactam in the empirical setting remains unknown.

Methods: Strains underwent genetic analysis evaluating *bla*$_{KPC}$ presence/production and MICs were determined. Four strains were assessed in an *in vitro*, one-compartment pharmacokinetic (PK)/pharmacodynamic (PD) model for 96 h. The following bolus dosing exposures were tested: 2.5 g of ceftazidime/avibactam every 8 h, 2 g of meropenem every 8 h, 1.25 mg/kg polymyxin B every 12 h, amikacin 'once-daily dosing' (peak of 70–80 mg/L), tigecycline at 200 mg ×1 dose followed by 100 mg every 12 h, and a drug-free growth control.

Results: Thirty *bla*$_{KPC}$-producing strains were evaluated; 97% of strains were ceftazidime/avibactam susceptible with MIC$_{50}$/MIC$_{90}$ values of 0.38/1.5 mg/L (range 0.032–16 mg/L). Two *K. pneumoniae* strains, one *Klebsiella oxytoca* strain and one *Citrobacter freundii* strain underwent further analysis in PK/PD models. Ceftazidime/avibactam displayed potent activity with a reduction of 4.23±0.42 cfu/mL from the initial inoculum at 96 h. Against susceptible isolates, amikacin displayed similar activity compared with ceftazidime/avibactam at 96 h, although this was not demonstrated against all strains. Polymyxin B produced comparable activity to ceftazidime/avibactam against two strains. Neither meropenem nor tigecycline produced effective killing and were comparable to the drug-free growth control at 96 h.

Conclusions: *bla*$_{KPC}$-producing organisms demonstrated susceptibility to ceftazidime/avibactam and bactericidal activity was observed in the PK/PD model. Based on these data, ceftazidime/avibactam is a valuable agent for treating KPC-producing organisms and should be considered for treatment of infections caused by these pathogens.

Introduction

Gram-negative bacteria demonstrating resistance to antibacterial agents represent a significant public health burden worldwide. The emergence of isolates of Enterobacteriaceae producing carbapenemases, most frequently harbouring the *Klebsiella pneumoniae* carbapenemase (KPC) enzyme in the USA, as well as other countries, is of significant concern due to their ability to render all β-lactams ineffective. Fortunately, many of these carbapenem-resistant Enterobacteriaceae (CRE) are colonizers or are pathogenic in infections with inoculums that are relatively easy to treat, such as urinary tract infections.[1,2] Mortality rates for more severe higher inoculum infections, however, including bacteraemia, are high and range ∼20%–70%.[3–6]

The poor chance of survival observed among patients with invasive Gram-negative infections producing KPCs is likely attributed to both significant delays in time to appropriate therapy and the lack of effective antimicrobial options, given that the majority of strains display resistance to the fluoroquinolones and carbapenems. While, polymyxins, aminoglycosides and tigecycline remain the most active *in vitro* options to treat these pathogens, these

drugs are hindered by their inefficiency as monotherapeutic options and/or toxicity.[7,8]

The FDA approved ceftazidime/avibactam for the treatment of complicated intra-abdominal infections in combination with metronidazole, complicated urinary tract infections and hospital-acquired bacterial pneumonia and ventilator-associated pneumonia. While ceftazidime is a well-described 'third-generation' β-lactam, avibactam represents a unique class of non-β-lactam β-lactamase inhibitors, which demonstrates inhibitory activity against Ambler class A, C and some D β-lactamases.[9] *In vitro* studies demonstrated potent activity of ceftazidime/avibactam against KPC-producing strains with reported $MIC_{50/90}$ values of 1/2 mg/L in one study and 0.5/2 mg/L in another, both well below the approved breakpoint of 8/4 mg/L.[10–13]

Early observational studies reporting experiences with ceftazidime/avibactam have been encouraging, but data are limited to small sample sizes. Additionally, ceftazidime/avibactam was frequently utilized as salvage therapy or in combination with other antimicrobials.[14–16] The objective of this study was to evaluate the activity of ceftazidime/avibactam alone compared with standard-of-care agents in an *in vitro* pharmacokinetic (PK)/pharmacodynamic (PD) model simulating bacteraemia with KPC-producing pathogens.

Materials and methods

Bacterial strains

A total of 30 clinical strains from the Detroit Medical Center underwent genetic analysis to confirm bla_{KPC} production. bla_{KPC} was amplified using established primers and each amplicon was sequenced.[17] Four representative KPC strains (two *K. pneumoniae*, one *Klebsiella oxytoca* and one *Citrobacter freundii*) were utilized for *in vitro* PK/PD modelling experimentations.

Antimicrobials

Avibactam was provided by its manufacturer (Allergan, Parsippany, NJ, USA). Ceftazidime, meropenem, amikacin, polymyxin B and tigecycline were commercially purchased (Sigma Chemical Co., St Louis, MO, USA).

Antimicrobial susceptibility testing

In vitro antimicrobial susceptibility testing was performed on all 30 strains. Vitek®2 or Microscan was utilized when possible to determine MIC. For ceftazidime/avibactam, polymyxin B and tigecycline, Etests were utilized following methodology according to the manufacturer. Antibiotic containing plates for resistance screening used brain heart infusion agar (Difco, Detroit, MI, USA).

In vitro PK/PD model

An *in vitro*, one-compartment PK/PD model with a 250 mL capacity and input/outflow ports was used. The apparatus was prefilled with medium (Mueller–Hinton broth) and antimicrobials were administered as boluses over a 96 h time period. A starting inoculum of $\sim 1 \times 10^6$ cfu/mL was targeted for each experiment. Fresh medium was continuously supplied and removed from the compartment along with the drug via a peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, IL, USA) at an appropriate rate to simulate the average human half-lives of the antimicrobials or at the longest half-life for the drug-free growth control. The apparatus was maintained at 37°C throughout the duration of experimentation. All experiments were performed in duplicate.

The antimicrobial regimen simulations evaluated as bolus doses were as follows: 2.5 g of ceftazidime/avibactam every 8 h (fC_{max} 93.2/13.6 mg/L, average $t_{1/2}$ 2.7 h),[18–20] 2 g of meropenem every 8 h (fC_{max} 110 mg/L, average $t_{1/2}$ 1 h),[21,22] 1.25 mg/kg polymyxin B every 12 h (fC_{max} 6.13 mg/L, average $t_{1/2}$ 6 h),[23,24] amikacin once-daily dosing to achieve a peak of 70–80 mg/L (average $t_{1/2}$ 2 h),[24–27] tigecycline at 200 mg × 1 dose followed by 100 mg every 12 h (fC_{max} 0.3 mg/L, average $t_{1/2}$ 42 h)[28] and a drug-free growth control.

PD analysis

Samples were removed at 0, 4, 8, 24, 32, 48, 72 and 96 h and serially diluted in cold 0.9% sodium chloride. Bacterial counts were determined by spiral plating appropriate dilutions using a Whitley automatic spiral plater (DW Scientific, Shipley, West Yorkshire, UK). Tryptic soy agar plates were incubated at 37°C for 24 h before colonies were counted. Antimicrobial killing was demonstrated via plotting mean ± SD colony counts ($\log_{10}$ cfu/mL) versus time with the lower limit of detection being 1 $\log_{10}$ cfu/mL. Bactericidal activity was defined as ≥3 $\log_{10}$ cfu/mL reduction from baseline.

PK analysis

PK samples were obtained, through the injection port of each model at appropriate timepoints throughout the model for verification of target antibiotic concentrations. All samples were stored at −80°C until ready for analysis. Ceftazidime/avibactam concentrations were sent out to Keystone Bioanalytical, Inc. for LC-MS analysis.[29] All other drug concentrations were determined by bioassay, as previously described.[30–32] In brief, blank 0.635 cm discs were spotted with 10 μL of the standards or samples. Each standard was tested in duplicate by placing the disc on antibiotic medium agar plate no. 11, which was inoculated with a 0.5 McFarland suspension of the test organism. Plates were incubated for 24 h at 37°C at which time the zone sizes were measured using a protocol reader (Protocol; Microbiology International, Frederick, MD, USA). The half-life, AUC_{0-24}, peak concentrations and time above MIC were determined utilizing PK Analyst software (version 1.10; MicroMath Scientific Software, Salt Lake City, UT, USA) using the linear trapezoidal method.

Resistance

Emergence of resistance (treatment emergent resistance) was evaluated daily by plating 100 μL samples from the model on plates supplemented at a concentration 3× the MIC of the tested antimicrobial. Plates were examined for growth after 48 h of incubation at 37°C. Resistant colonies growing on screening plates were evaluated by Etest or broth microdilution methods to determine the MIC.

Statistical analysis

Changes in cfu/mL at 96 h were compared by one-way analysis of variance with Tukey's *post hoc* test. All statistical analyses were performed using SPSS Statistical Software (Release 22.0; IBM Corp., Armonk, NY, USA).

Results

Susceptibility testing

The MIC results for the isolates evaluated are summarized in Table 1. A total of 29 of 30 (97%) of isolates were ceftazidime/avibactam susceptible with MIC_{50} and MIC_{90} values of 0.38 and 2 mg/L (range 0.032–16 mg/L) despite only five of the isolates being ceftazidime susceptible. Strains 6R, 11R, 4299 and 4329 were evaluated in the PK/PD models. These strains were MDR and

Table 1. Susceptibilities (mg/L) of study organisms

Isolate	Organism	CZA	CAZ	MEM	AMK	TOB	CIP	LVX	SXT	TGC	PMB
1	*K. pneumoniae*	0.25	4	>8	≤2	≤0.5	≤0.5	≤1	≤1	0.19	0.125
3	*Escherichia coli*	0.5	≤0.5	>8	≤8	≤2	≤0.5	≤1	≤1	2	0.19
4	*K. pneumoniae*	0.38	>16	>8	>32	>8	>2	>4	>2	1.5	0.38
6	*K. oxytoca*	0.94	>16	>8	>32	>8	>2	>4	>2	3	0.38
10	*K. pneumoniae*	0.38	>16	>8	16	>8	>2	>4	>2	3	0.25
12	*K. pneumoniae*	0.75	>16	>8	32	>8	>2	>4	>2	1.5	0.38
14	*K. pneumoniae*	0.064	>16	>8	16	>8	>2	>4	>2	4	0.38
18	*K. pneumoniae*	0.25	>16	>8	32	>8	>2	>4	>2	1	0.38
20	*K. pneumoniae*	0.75	>16	>8	32	>8	>2	>4	>2	3	0.75
21	*K. pneumoniae*	0.064	>16	>8	≤2	>8	>2	>4	≤1	3	0.38
22	*K. pneumoniae*	0.032	≤1	>8	≤2	≤0.5	≤0.5	≤1	≤1	2	0.38
24	*K. pneumoniae*	0.75	>16	8	16	>8	>2	>4	>2	3	0.38
30	*K. pneumoniae*	2	>16	>8	32	>8	>2	>4	>2	3	0.5
34	*K. pneumoniae*	0.75	>16	>8	32	>8	>2	>4	>2	2	0.5
35	*K. pneumoniae*	0.38	>16	>8	≤4	>8	>2	>4	>2	3	0.5
36	*K. pneumoniae*	3	>16	>8	32	>8	>2	>4	>2	0.38	1
38	*K. pneumoniae*	2	>16	>8	32	>8	>2	>4	>2	2	0.5
43	*K. pneumoniae*	0.5	>16	>8	32	>8	>2	>4	>2	3	0.38
45	*K. pneumoniae*	0.125	>16	>8	32	>8	>2	>4	>2	4	0.38
46	*E. coli*	1	>16	>8	16	>8	>2	≤1	>2	3	2
4091	*K. pneumoniae*	0.047	>16	≥16	≤2	>16	≥4	>4	>320	0.75	0.19
4164	*K. oxytoca*	16	>16	>8	≤2	4	>2	2	>40	1.5	128
4234	*K. pneumoniae*	0.125	4	≥16	≤2	≤1	1	≤1	40	2	0.38
4299	*C. freundii*	0.19	>16	≥16	≤2	8	≥4	>4	>320	6	0.38
4329	*K. pneumoniae*	0.5	>16	≥16	4	≤1	≥4	>4	≤20	1.5	0.75
1R	*K. pneumoniae*	1.5	>16	>8	>32	≤1	>2	>4	>2	1.5	1
3R	*K. pneumoniae*	0.047	>16	>8	32	>8	>2	>4	>2	3	0.5
5R	*K. pneumoniae*	0.38	4	>8	>32	2	≤0.5	≤1	≤1	1.5	0.75
6R	*K. oxytoca*	0.75	>16	>8	32	>8	>2	>4	>2	1	0.5
11R	*K. pneumoniae*	0.19	>16	>8	>32	>8	>2	>4	>2	3	0.38

CZA, ceftazidime/avibactam; CAZ, ceftazidime; MEM, meropenem; AMK, amikacin; TOB, tobramycin; CIP, ciprofloxacin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole; TGC, tigecycline; PMB, polymyxin B.

were not susceptible to ceftazidime, meropenem or ciprofloxacin. Two strains (4299 and 4329) were susceptible to amikacin with MICs of ≤2 and 4 mg/L, respectively. Only one strain (4329) was susceptible to trimethoprim/sulfamethoxazole. All four strains were susceptible to ceftazidime/avibactam, polymyxin B and tigecycline.

In vitro PK/PD model

The observed fC_{max} and $t_{1/2}$ values for amikacin were 74.6±5.9 mg/L (target 70 mg/L) and 2.12±0.2 h (target 2 h). The observed fC_{max} and $t_{1/2}$ values for meropenem were 104±7.4 mg/L (target 110 mg/L) and 0.96±0.2 h (target 1 h). The observed fC_{max} and $t_{1/2}$ values for polymyxin B were 6.2±0.9 mg/L (target 6.13 mg/L) and 5.92±0.5 h (target 6 h). The observed fC_{max} and $t_{1/2}$ values for tigecycline were 0.4±0.1 mg/L (target 0.3 mg/L) and 38.4±4.1 h (target 42 h). The observed fC_{max} and $t_{1/2}$ values for ceftazidime/avibactam were 93.1±5.14/12.9±2.07 mg/L (target 93.2/13.6 mg/L) and 2.5±0.25 h (target 2.7 h), respectively.

The changes in $\log_{10}$ cfu/mL for the tested regimens against the four strains are displayed in Figure 1. Against all strains, ceftazidime/avibactam demonstrated bactericidal activity with an average reduction of 4.23±0.42 cfu/mL from the starting inoculum within 8–24 h. By 96 h, ceftazidime/avibactam was statistically the most effective antimicrobial against three of the four strains ($P < 0.01$). However, against strain 4299, both amikacin and polymyxin B were comparable to ceftazidime/avibactam. Amikacin displayed potent killing (3.85±0.72 reduction in starting inoculum) within the first 8 h in all strains. However, regrowth was noted at 24 h in amikacin non-susceptible strains 6 R and 11 R (MICs 32 and ≥32 mg/L, respectively). Similar to amikacin, polymyxin B demonstrated bactericidal activity within 8 h (3.08±0.84 reduction in starting inoculum) and continued throughout the duration of the experiment (3.78±1.45 reduction in starting inoculum) against two of the strains (4299 and 4329). However, in the other two strains (6 R and 11R), sustained killing was not observed. Tigecycline reduced the initial inoculum within the first 4–8 h but regrowth was noted in all strains by 24 h. As expected due to meropenem resistance in all strains (MICs >8 mg/L for four strains

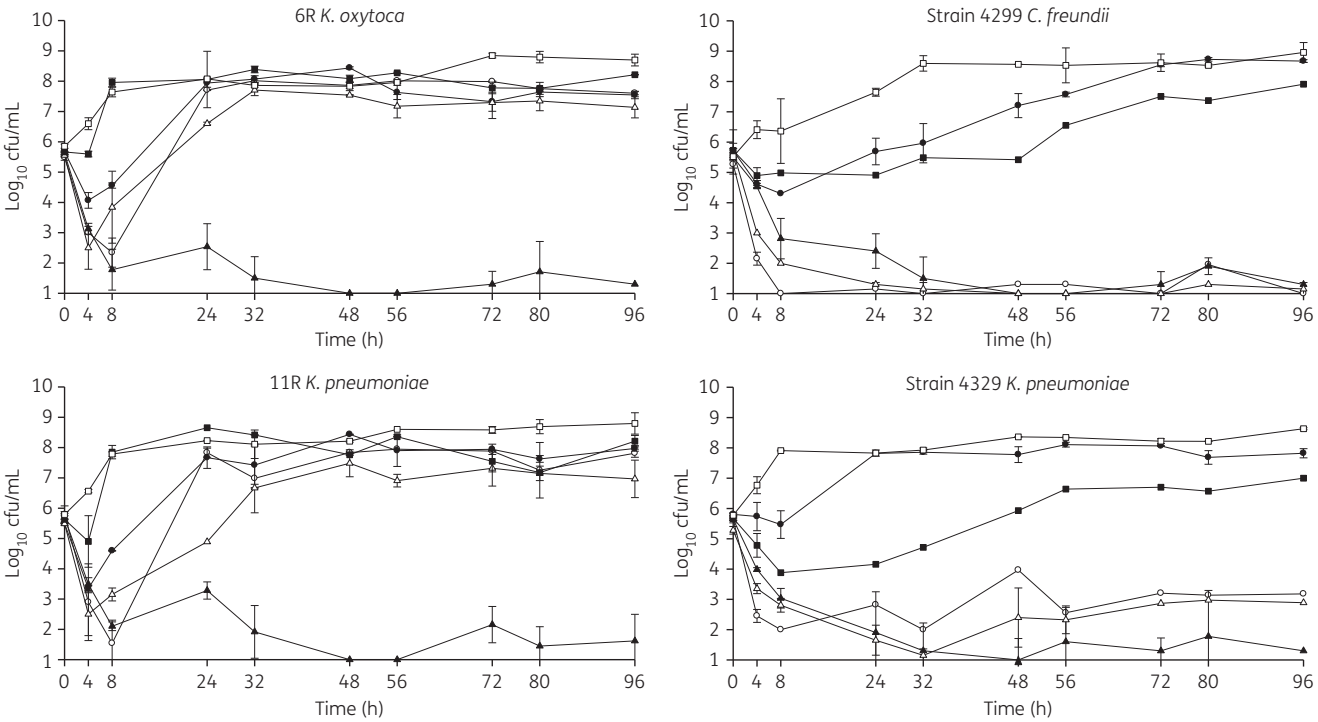

Figure 1. PK/PD graphs for four study pathogens in *in vitro* models. Open circles, amikacin; filled triangles, ceftazidime/avibactam; filled circles, meropenem; open triangles, polymyxin B; filled squares, tigecycline; open squares, drug-free growth control.

evaluated in PK/PD models), meropenem activity at 96 h was comparable to the drug-free growth control in all strains evaluated, with regrowth occurring as early as 8 h in two of the four strains and by 24 h in the remaining two.

Resistance emergence

Isolates with MICs that were higher than the baseline MICs were not detected on resistance screening plates.

Discussion

Studies have demonstrated that inappropriate empirical coverage, particularly for bloodstream infections, yields higher mortality compared with appropriate therapy.[33] In light of the increasing presence and degree of Gram-negative antimicrobial resistance, clinicians face an increasing challenge when attempting to cover empirically the most likely pathogens with an agent that has retained activity against those organisms. In centres with a high prevalence of CRE, this is particularly challenging, as effective and safe treatment options are limited.

In this evaluation, in an *in vitro* model, we demonstrated that initial monotherapy with ceftazidime/avibactam displays potent bactericidal activity against CRE regardless of resistance to other classes of antimicrobials. Bactericidal activity was noted in all strains evaluated and susceptibility was restored despite high prevalence to ceftazidime resistance. While tigecycline displayed an initial kill in the first 4–8 h of experimentation, it was not anticipated that this agent would produce a sustained killing effect due to low concentrations utilized for the simulation of a bloodstream infection. However, this agent may still be beneficial for other infection types where the drug achieves high concentrations. In two strains (6R and 11R) treated with polymyxin B, regrowth was noted despite no development of resistance. However, this could be due to limitations of Etest methodology for the polymyxins with up to 20% of isolates being falsely reported as susceptible.[34,35] Additionally, there is potential that resistant mutants could have been pumped out of the model system due to the type of *in vitro* modelling utilized with high flow rates.

Given the challenges with treating CRE, ceftazidime/avibactam is a viable therapeutic option. However, in clinical settings where ceftazidime/avibactam has been employed, it is frequently prescribed in combination or as salvage therapy. In a multicentre study evaluating clinical outcomes in 60 CRE-infected patients treated with ceftazidime/avibactam, microbiological cure and clinical success were observed in 53% and 65% of patients, respectively.[14] The majority of these patients demonstrated a high degree of acute illness with invasive infections. What makes the impact of ceftazidime/avibactam particularly difficult to interpret is that roughly half of the patients received concomitant therapy most commonly with an aminoglycoside, polymyxin and/or tigecycline.

Several studies evaluated ceftazidime/avibactam for salvage therapy with high cure rates (74%) and low mortality (8%).[15,36] However, it is important to note that combination therapy was common and occurred in 66%–85% of patients. One study reported outcomes in 37 CRE-infected patients treated with ceftazidime/avibactam demonstrated similar clinical success in 59% of patients.[14] Unlike the previously mentioned evaluations, the majority of patients in this evaluation received monotherapy (70%) with a clinical success rate of 58%.

There are several limitations of the current study. First, experiments were only conducted for 96 h with only four strains. While no ceftazidime/avibactam resistance development was noted during this time, organisms can develop resistance after 96 h of therapy. However, development of resistance after 96 h has only been described in three ceftazidime/avibactam-treated patients to date.[37] Many patients receive Gram-negative antimicrobial therapy prior to the identification of a carbapenem-resistant organism. In our experiment, isolates were not subjected to antimicrobial exposure prior to the receipt of ceftazidime/avibactam. Combination therapy is often utilized to treat these organisms and this was not assessed. Lastly, all analyses were conducted based upon simulation of normal renal clearance; therefore, altered killing may be observed in patients with decreased or increased renal function.

In conclusion, in this *in vitro* model, ceftazidime/avibactam was efficacious against carbapenem-resistant organisms, specifically those producing KPC enzymes. In patients at high risk for CRE infection caused by KPC production, ceftazidime/avibactam monotherapy appears to be an effective empirical therapeutic agent although future studies with combination therapy are still warranted.

Acknowledgements

We thank Allergan for providing avibactam powder.

Funding

This work was supported by Forest Laboratories now owned by Allergan. R. A. B. receives grant funding from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award numbers R01AI100560, R01AI063517, R21AI114508 and R01AI072219. This study was supported in part by funds and/or facilities provided by the Cleveland Department of Veterans Affairs, award number 1I01BX001974 to R. A. B. from the Biomedical Laboratory Research & Development Service of the VA Office of Research and Development and the Geriatric Research Education and Clinical Center VISN 10.

Transparency declarations

K. E. B. has received grant funding from Allergan. J. M. P. is a consultant, speaker and/or receives grant funding from Allergan, Merck, Medicines Company, Achaogen, Shionogi and Zavante. K. S. K. has received grant funding from Merck and Allergan and consults for Merck, Allergan, Melinda, Achaogen, Shionogi and Zavante. H. D. W. and R. A. B.: none to declare.

Disclaimer

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