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NCase report

Suboptimal drug exposure leads to selection of different subpopulations of ceftazidime-avibactam-resistant *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* in a critically ill patient

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ABSTRACT

Objectives: Ceftazidime-avibactam (CAZ-AVI) is a promising novel agent with activity against carbapenem-resistant *Enterobacteriaceae*. Here, we describe the dynamic evolution of a *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) infection in a critically ill patient treated with CAZ-AVI-tigecycline combination therapy.

Methods: Whole-genome sequencing was performed on longitudinal intrapatient KPC-Kp strains isolated from different sites during CAZ-AVI treatment. The pharmacokinetic/pharmacodynamic (PK/PD) analysis was performed on the basis of therapeutic drug monitoring of ceftazidime.

Results: The development of resistance due to mutations in the blaKPC gene was observed in KPC-Kp strains isolated from bronchoalveolar lavage and blood during CAZ-AVI treatment. PK/PD analysis demonstrated that during the first days of treatment CAZ- AVI blood exposure was suboptimal (steady-state concentration/minimum inhibitory concentration ratio 2.85). Of note, the low antibiotic pressure may have selected hybrid subpopulations harboring bla_{KPC-3} and T243M mutation in KPC-Kp isolated from bronchoalveolar lavage and D179Y mutation in those isolated from blood.

Conclusion: These results suggest the high adaptability of KPC to CAZ-AVI due to the rapid evolution of resistance and highlight the importance of identifying the optimal PK/PD target to prevent such an event from occurring again in a critically ill patient with pneumonia due to KPC-Kp.

Introduction

Ceftazidime-avibactam (CAZ-AVI) is currently endorsed as a first-line treatment for carbapenem-resistant *Enterobacteriaceae*-related infections due to its *in vitro* activity and safety profile and the lack of other feasible options (Zhanel et al. 2013). However, the efficacy of CAZ-AVI-based treatment has been affected by the recent emergence of resistant strains (Shields et al. 2018; Gaibani et al. 2018). Suboptimal drug exposure is hypothesized to be responsible for selection for CAZ-AVI resistance, especially among critically ill patients (Adembri et al., 2020). Here, we describe the stepwise evolution of CAZ-AVI-resistant *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) subpopulations that occurred in a patient with a bloodstream infection who experienced suboptimal pharmacokinetic exposure during CAZ-AVI treatment.

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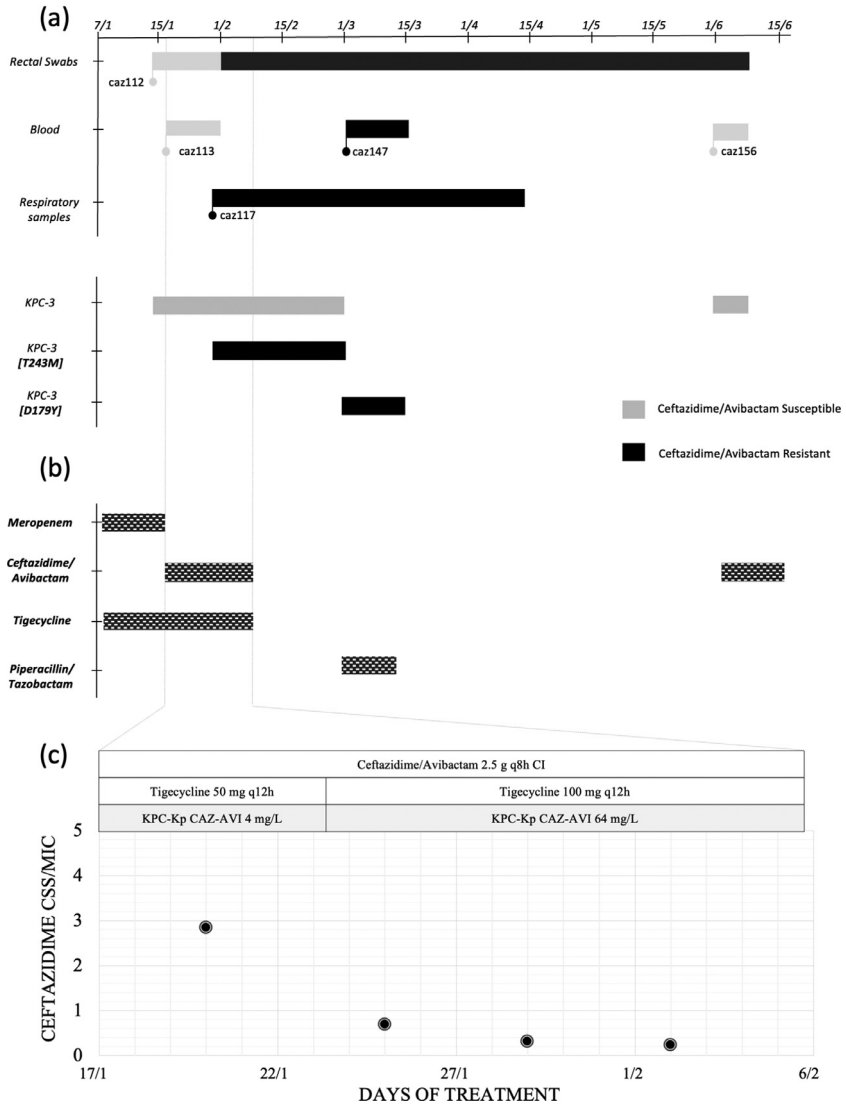

Figure 1. Timeline of sampling, antimicrobial treatments and ceftazidime concentrations.
(a) *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) isolations from different sample types (upper part) and presence of different bla_{KPC} (bottom part); (b) Antimicrobial treatment (striped bars); (c) Ratio of free ceftazidime serum concentration and minimum inhibitory concentration for ceftazidime-avibactam to KPC-Kp isolate)

Case report

A 77-year-old man was admitted to hospital on 7 January 2021 for progressive jaundice. Abdominal imaging revealed a malignant lesion in the distal biliary tract. After 4 days, the patient underwent spleno-pancreatectomy and biliary-digiunal anastomosis. Surveillance rectal swabs revealed that the patient was colonized by a KPC-Kp strain (Figure 1). Antimicrobial susceptibility testing was performed as previously described (Gaibani et al., 2018). The minimum inhibitory concentration (MIC) results were interpreted following the European Committee on Antimicrobial Susceptibility Testing's clinical breakpoints v.11.0 (https://www.eucast.org/clinical_breakpoints). Antimicrobial susceptibility results showed that the KPC-Kp (named CAZ112) was susceptible to CAZ-AVI (MIC 2 mg/L), aminoglycosides and colistin, and resistant to carbapenem. Whole-genome sequencing (WGS) analysis was performed, as previously described (Gaibani et al. 2018), to clarify the basis of antimicrobial resistance. Briefly, sequencing was performed by the Illumina Iseq100 platform, genomes assembled with Spades v.3.15.3, and antimicrobial resistance and sequence type (ST) investigated using the Center for Genomic Epidemiology server, as previuously described (Gaibani et al., 2018). WGS results showed that CAZ112 belonged to ST101 and harbored wild-type OmpK36 and OmpK37 porins, truncated ompK35, and blaKPC-3 bla_{SHV-28}, and $bla_{SHV-106}$ ß-lactamase (Table 1). The postoperative period was complicated by a biliary fistula and fever; therefore, 5 days after his first surgery, the patient underwent surgical revision. Empirical treatment based on high doses of continuous infusion meropenem plus tigecycline was started. Blood cultures collected 4 days after empirical treatment yielded a KPC-Kp strain (CAZ113) that was highly resistant to meropenem and aminoglycosides but was susceptible to colistin and CAZ-AVI (4 mg/L) and had low MIC for tigecycline (≤1 mg/L). WGS analysis demonstrated that CAZ113 KPC-Kp belonged to ST101 and shared with CAZ112 the same susceptibility genes pattern, plasmid replicon types and porin genes content. According to antimicrobial susceptibility results, antibiotic therapy was switched from meropenem plus tigecycline to CAZ-AVI plus tigecycline (Figure 1). After 10 days of CAZ-AVI-based treatment, the patient developed *ab ingestis* pneumonia caused by a new KPC-Kp

Table 1
Phenotypic and genotypic characteristics of KPC-producing *Klebsiella pneumoniae* strains.

Isolate	Source	MIC (mg/L)											Genetic Determinant Resistances					Porins			Plasmid_replicons (InC)
		CST	MEM	CAZ	CFD	IPM/REL	CAZ/AVI	MER/VAB	GEN	AMK	TGC	PIP/TAZ	ST	Carbapenemase (Frequency)	Beta-lactams	Aminoglycosides	Fluoroquinolones	Fosfomycin	*OmpK35*	*OmpK36*	*OmpK37*	
CAZ112	Rectal swab	0,5	**256**	**>= 256**	4	0,5	2	1	<=2	<=8	<=1	**>= 128**	101	*bla*$_{KPC-3}$ *(100)*	*bla*$_{SHV-28}$ [a], *bla*$_{SHV-106}$ [a]	-	*oqxA, oqxB,*	*fosA*	truncated at aa 62	WT	WT	Col440II, ColRNAI, IncFIA(HI1), IncFIB(K), IncFIB(pNDM-Mar), IncFII(K), IncR
CAZ113	Blood	0.5	**256**	**>= 256**	8	0,5	4	1	**>4**	**>16**	<=1	**>= 128**	101	*bla*$_{KPC-3}$ *(100)*	*bla*$_{SHV-28}$ [a], *bla*$_{SHV-106}$ [a]	*armA*	*oqxA, oqxB,*	*fosA*	truncated at aa 62	WT	WT	Col40II, ColRNAI, IncFIA(HI1), IncFIB(K), IncFIB(pNDM-Mar), IncFII(K), IncR
CAZ117	Bronchoalveolar aspirate	0.5	**32**	**>= 256**	4	1	**64**	2	**>4**	**>16**	<=1	**>= 128**	101	*bla*$_{KPC-3}$ *(55%)* *bla*$_{KPC-3}$ *[T243M] (45%)*	*bla*$_{SHV-28}$ [a], *bla*$_{SHV-106}$ [a]	*armA*	*oqxA, oqxB*	*fosA*	truncated at aa 62	WT	WT	Col 40II, ColRNAI, IncFIA(HI1), IncFIB(K), IncFIB(pNDM-Mar), IncFII(K), IncR
CAZ147	Blood	0,5	2	**>= 256**	**16**	1	**256**	2	**>4**	**>16**	<=1	<=8	101	*bla*$_{KPC-3}$ *[D179Y] (100)*	*bla*$_{SHV-28}$ [a], *bla*$_{SHV-106}$ [a]	*armA*	*oqxA, oqxB*	*fosA*	truncated at aa 62	WT	WT	Col40II, ColRNAI, IncFIA(HI1), IncFIB(K), IncFIB(pNDM-Mar), IncFII(K), IncR
CAZ156	Blood	0,25	**256**	**>= 256**	8	1	4	1	**>4**	**>16**	<=1	**>= 128**	101	*bla*$_{KPC-3}$ *(100)*	*bla*$_{SHV-28}$ [a], *bla*$_{SHV-106}$ [a]	*armA*	*oqxA, oqxB*	*fosA*	truncated at aa 62	WT	WT	Col40II, ColRNAI, IncFIA(HI1), IncFIB(K), IncFIB(pNDM-Mar), IncFII(K), IncR

Abbreviations: CST, colistin; ETP, Ertapenem; MEM, meropenem; CAZ, ceftazidime; CFD, cefiderocol; IPM/REL, imipenem/relebactam; CAZ/AVI, ceftazidime/avibactam; MER/VAB, meropenem/vaborbactam GEN, gentamicin; AMK, amikacin; TGC, tigecycline; ST, sequence type; WT, wild type.
[a] $\geq$ 99% identity
Resistance are highlighted in bold.

strain (CAZ117). The antimicrobial susceptibility pattern revealed that CAZ117 was resistant to meropenem and CAZ-AVI (64 mg/L), with susceptibility to colistin and a low MIC value for tigecycline. WGS demonstrated that CAZ117 was composed of a hybrid subpopulation harboring wild-type and mutated bla_{KPC-3} genes; 55% of aligned Illumina reads obtained from CAZ117 mapped to the wild type bla_{KPC-3} gene, while 45% displayed a T243M mutation (Table 1).

With antimicrobial options limited, the same antimicrobial treatment was continued, and the dose of tigecycline doubled to ensure better exposure. The patient had a favorable outcome, and antibiotic treatment was discontinued on 2 February. However, after 1 month, the patient's general condition deteriorated, and empirical treatment with piperacillin-tazobactam was introduced. Blood cultures yielded a KPC-Kp strain (CAZ147) resistant to CAZ-AVI (256 mg/L), intermediate to piperacillin-tazobactam (16 mg/L) and susceptible to meropenem (2 mg/L). WGS demonstrated that CAZ147 shared a similar susceptibility genes pattern to previous clinical isolates (except for bla_{KPC-3} gene), showing 100% of aligned reads with the D179Y mutation ($ibla_{KPC-31}$) (Table 1). Therefore, piperacillin-tazobactam was confirmed as the treatment. After 10 days, the patient's clinical condition improved, and antibiotic treatment was definitively discontinued.

Almost 3 months later, the patient was newly admitted to the hospital due to a gradual worsening of clinical condition, fever and abdominal pain. Blood cultures showed the growth of a KPC-Kp (CAZ156) with restored susceptibility to CAZ-AVI (4 mg/L) and resistance to meropenem. WGS showed that the CAZ156 strain harbored the same antimicrobial susceptibility gene pattern to KPC-Kp clinical strains harboring bla_{KPC-3} gene and identical porin and plasmids contents to all previous strains (Table 1). Despite prompt empirical treatment with CAZ-AVI at hospital admission, the patient died on 8 June due to a fatal arrhythmia.

In order to investigate the dynamics of genetic variations occurring in KPC-Kp during CAZ-AVI-based treatment, we performed a comparative WGS analysis based on core genome single-nucleotide polymorphism (SNP). Analysis showed that the genome of CAZ117, CAZ147 and CAZ156 strains differed by 25, 32 and 34 SNPs with that of CAZ113 used as the reference.

Pharmacokinetic/pharmacodynamic (PK/PD) analysis

CAZ-AVI was administered at the dosage of 2.5 g at interval of 8h by continuous infusion after a loading dose of 2.5 g. Therapeutic drug monitoring performed 72 hours after starting treatment showed that the ceftazidime serum concentration was 11.4 mg/L; the KPC-Kp strain isolated from blood cultures had a MIC of 4 mg/L; therefore, the resulting steady-state concentration (C_{ss})/MIC ratio was 2.85. The measured creatinine clearance was 83 mL/min. Pharmacokinetic analysis showed that ceftazidime clearance (21.9 L/h) in our patient was more than double that previously observed in healthy volunteers (Merdjan et al., 2015). We attributed this to an unrecognized augmented renal clearance, a state that may commonly occur in critically ill patients during the first phase of sepsis (Cook and Hatton-Kolpek, 2019). Ceftazidime serum concentration was reassessed on day 8, 24 hours after the patient developed septic shock due to aspiration pneumonia. Under the same CAZ-AVI daily dosage, ceftazidime C_{ss} was increased to 44 mg/L due to worsening renal function caused by sepsis-related multi-organ failure (measured creatinine clearance, 37.7 mL/min). However, the C_{ss}/MIC ratio worsened to 0.69, considering that the KPC-Kp isolated from the bronchoalveolar lavage was CAZ-AVI resistant with a MIC of 64 mg/L. The following therapeutic drug monitoring reassessments, performed on day 12 and 16, showed ceftazidime C_{ss} of 20.2 and 15 mg/L, resulting in C_{ss}/MIC of 0.32 and 0.23, respectively; this was also due to rapid recovery from acute kidney injury. The measured creatinine clearance on day 12 and 16 was 68 and 64 mL/min, respectively.

4. Discussion

Here we described the emergence of CAZ-AVI -resistant KPC-Kp strains in a critically ill patient likely due to suboptimal drug exposure. PK/PD analysis demonstrated that suboptimal CAZ-AVI exposure during the first days of treatment could have induced the development of resistant subpopulations harboring mutated bla_{KPC} gene in comparison with the parental strain. Indeed, genome comparison of longitudinal clinical strains demonstrated that few mutations differentiated KPC-Kp isolates and that emerging rapid mutation within the bla_{KPC} gene was linked to CAZ-AVI resistance in strains. These data agree with previous studies demonstrating that CAZ-AVI-based antibiotic treatment is the main cause of the development of resistance (Shields et al. 2018; Gaibani et al. 2018).

To date, data reporting the optimal dosing regimens for CAZ-AVI in critically ill patients are scarce (Hites, 2021). However, emerging clinical data suggest that more aggressive PK/PD targets, up to Css/MIC of 4–5, may not only improve clinical efficacy but also suppress the emergence of resistance (Sumi et al., 2019; Heffernan et al. 2018; Tam et al. 2017). In this context, a PK/PD target of 100%$fT_{>4xMIC}$ has been suggested to suppress the emergence of resistance (Delattre et al., 2017; Li et al., 2019). In our clinical case, underexposure to CAZ-AVI probably induced the development of different mutations linked to CAZ-AVI-resistant strains isolated from different sites. The CAZ-AVI-based regimen reached a C_{ss}/MIC of 2.85 during the first days of therapy and resulted in suboptimal drug exposure and subsequent isolation of a KPC-Kp strain resistant to CAZ-AVI and carbapenem from bronchoalveolar lavage.

Of note, WGS demonstrated that KPC-Kp resistant both to CAZ-AVI and meropenem comprised complex subpopulations carrying different bla_{KPC} alleles responsible for the hybrid phenotype. These data agree with previous WGS studies demonstrating that a specific mutation in the bla_{KPC} gene is responsible for the CAZ-AVI-resistance phenotype associated with reverted carbapenem-susceptibility, and those mixed subpopulations are associated with hybrid phenotypes in longitudinal intrapatient samples (Shields et al. 2018; Gaibani et al., 2018).

Conclusion

This case report documented the dynamic evolution of breakthrough resistance to CAZ-AVI in *Klebsiella pneumoniae* strains due to suboptimal drug exposure during CAZ-AVI treatment and confirmed the high capability of adaptation that KPC-Kp may have to this drug. Further evaluations are warranted to understand the appropriate PK/PD target for optimal CAZ-AVI treatment of KPC-Kp pneumonia in critically ill patients.

Conflicts of interest

The authors declare no conflicts of interest.

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Ethical approval

The study was conducted in the context of routine clinical practice. The study was conducted in accordance with the Declaration of Helsinki.

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