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Short Communication

In vitro interaction of ceftazidime–avibactam in combination with different antimicrobials against KPC-producing *Klebsiella pneumoniae* clinical isolates

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ABSTRACT

Objectives: Combination therapy has been recommended when using ceftazidime–avibactam (CAZ–AVI) for the treatment of KPC-producing *Klebsiella pneumoniae* (KPC-Kp), but the optimal combination is unknown. Six common antimicrobial agents (ertapenem, imipenem, meropenem, gentamicin, tigecycline, and ciprofloxacin) were evaluated for synergy with the recently approved cephalosporin–β-lactamase inhibitor combination CAZ–AVI in this study.
Methods: Different antimicrobial combinations were tested against 13 KPC-Kp, including CAZ–AVI-susceptible ($n = 11$) and resistant ($n = 2$) clinical isolates. In vitro interactions of CAZ–AVI with different antimicrobials were tested using the gradient synergy test. Changes in the minimum inhibitory concentration (MIC) value were interpreted using the fractional inhibitory concentration (FIC) index and susceptible breakpoint index (SBPI).
Results: The combination of CAZ–AVI with gentamicin or ciprofloxacin displayed no synergism against any of the KPC-Kp isolates, whereas synergistic activity was observed with imipenem and meropenem against all KPC-Kp isolates. Notably, CAZ–AVI reduced MICs for meropenem and imipenem below the resistance breakpoints against all strains. The SBPI analysis showed that CAZ–AVI in combination with imipenem achieved higher SBPI values than other CAZ–AVI-based combinations.
Conclusions: These data suggest that combinations of CAZ–AVI with imipenem may be considered a useful therapeutic option for the treatment of KPC-Kp infections.

Introduction

Over the last decade, the emergence of carbapenem-resistant *Klebsiella pneumoniae* has increased in frequency and it has disseminated worldwide (Pitout et al., 2015). Carbapenem resistance in *K. pneumoniae* is commonly due to the production of carbapenemase, mainly KPC, which is the most common enzyme in many countries (Munoz-Price et al., 2013). Infections caused by KPC-producing *K. pneumoniae* (KPC-Kp) are associated with poor clinical outcomes, which are directly attributable to the lack of effective antimicrobial treatment options. Given the limited treatment options available, combination antimicrobial therapy is considered the most viable therapeutic strategy for achieving maximal antimicrobial effects against KPC-Kp (Pitout et al., 2015).

Ceftazidime–avibactam (CAZ–AVI) has recently been reported to be effective in the treatment of bloodstream infections caused by carbapenemase-producing *Enterobacteriaceae* (Temkin et al. 2017; Wu et al., 2016). However, the initial promising results of monotherapy have been tempered by reports of the emergence of CAZ–AVI resistance during monotherapy (Shields et al. 2017, 2016). These observations highlight the urgent need to evaluate new strategies, including combination therapy, for the treatment of infections due to KPC-Kp (Shields et al. 2017).

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In this study, the activity of CAZ–AVI in combination with meropenem, ertapenem, imipenem, tigecycline, ciprofloxacin, and gentamicin was tested against 13 KPC-Kp strains, including two CAZ–AVI-resistant clinical isolates.

Materials and methods

The 13 non-duplicate *K. pneumoniae* strains analysed in this study were isolated between 2011 and 2017, from patients hospitalized in St. Orsola-Malpighi Hospital, Bologna, Italy. Antimicrobial susceptibility testing was performed using minimum inhibitory concentration (MIC) test strips (Liofilchem, Italy) and results were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017) clinical breakpoints v7.1. The MIC value for colistin was determined by broth microdilution method following EUCAST recommendations (EUCAST, 2016). The bla_{KPC} alleles and the mechanism of colistin resistance were determined by PCR followed by sequencing (Cannatelli et al., 2013). The genetic relationships among the different *K. pneumoniae* isolates were investigated by multilocus sequence typing (MLST) (Gaibani et al., 2014).

Synergy testing was performed by gradient diffusion method, as described previously (Gaibani et al. 2014). The fractional inhibitory concentration (FIC) index was calculated for each combination and results were interpreted as follows: synergy, FIC $\leq$ 0.5; indifferent, 0.5 > FIC $\leq$ 4; antagonism, FIC $\geq$ 4.

The susceptible breakpoint index (SBPI) was calculated as follows: [susceptible breakpoint A/MIC $A_{incombination}$] + [susceptible breakpoint B/MIC $B_{incombination}$] (Milne and Gould, 2010).

Results

As shown in Table 1, 15% (2/13) of isolates were resistant to CAZ–AVI (≥256 μg/ml), while 85% of KPC-Kp strains were susceptible (0.75 μg/ml to 2 μg/ml). Genetic analysis of colistin resistance showed disruption of the *mgr*B gene by different insertion sequence (IS) elements (i.e., ISKpn25 (ISL3 family) and ISKpn26 (IS5 family)) in all colistin-resistant strains. Moreover, all CAZ–AVI-resistant KPC-Kp strains possessed a D179Y mutation within the bla_{KPC-3} gene.

The results of synergy testing are shown in Table 1. CAZ–AVI in combination with gentamicin or ciprofloxacin was indifferent against all strains, whereas CAZ–AVI plus tigecycline was synergistic against 8% (1/13) of KPC-Kp isolates. In addition, double carbapenems (i.e., meropenem plus ertapenem) displayed synergistic activity against 31% (4/13) of KPC-Kp strains.

Synergy testing showed that CAZ–AVI in combination with meropenem and imipenem displayed a synergistic effect against all KPC-Kp isolates. At the same time, when CAZ–AVI was tested in combination with ertapenem, a synergistic effect was observed only against CAZ–AVI-susceptible *K. pneumoniae* strains (Table 1). Deeper examination of the synergy results showed that CAZ–AVI restored meropenem and imipenem susceptibility in the case of 50% (5/10) and 80% (8/10), respectively, of KPC-3-producing *K. pneumoniae* strains, while carbapenem susceptibility was not restored in the case of KPC-2-producers (**Supplementary Material**, Figure S1).

In order to evaluate the potency of these combinations, SBPI values were determined for all antimicrobial combinations against

Table 1
Summary of antimicrobial susceptibility and synergy testing results for ceftazidime–avibactam-susceptible and resistant KPC-producing *K. lebsiella pneumoniae* clinical isolates.

	Strain												
	Kp1	Kp2	Kp3	Kp4	Kp5	Kp6	Kp7	Kp8	Kp9	Kp10	Kp11	Kp12	Kp13
ESBL	bla_{SHV}	bla_{SHV}, bla_{TEM}	bla_{SHV},	bla_{SHV}, bla_{TEM}	bla_{SHV}, bla_{TEM}	bla_{SHV}, bla_{TEM}	bla_{SHV}, bla_{TEM}	bla_{SHV}, bla_{TEM}	bla_{SHV}, bla_{TEM}	bla_{SHV}, bla_{TEM}	bla_{SHV}, bla_{TEM}	bla_{SHV}, bla_{TEM}	bla_{SHV}, bla_{TEM}
bla_{KPC}	bla_{KPC-2}	bla_{KPC-2}	bla_{KPC-3}	bla_{KPC-3}	bla_{KPC-3}	bla_{KPC-3}	bla_{KPC-3}	bla_{KPC-3}	bla_{KPC-3}	bla_{KPC-3}	bla_{KPC-2}	bla_{KPC-3}[a]	bla_{KPC-3}[a]
MLST	ST258	ST258	ST512	ST258	ST258	ST258	ST512	ST258	ST554	ST307	ST101	ST1519	ST1519
Antimicrobial susceptibility (MIC, μg/ml)													
CAZ	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256
ETP	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	8	≥32
IPM	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	12	≥32	0.19	≥32
MEM	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	8	≥32
CAZ–AVI	1.5	1.5	2	1.5	1.5	2	0.75	1.5	0.75	1.5	1	≥256	≥256
GEN	1.5	1.5	2	2	2	1	1	1.5	2	0.75	≥256	2	2
TGC	0.19	0.75	1	1	1	1	1.5	2	2	12	0.25	1.5	1.5
CIP	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32
CST[b]	16	0.125	0.5	0.5	16	32	32	32	0.5	0.25	8	32	0.5
Antimicrobial combination (FIC index)													
MEM + CAZ	2	2	1.37	1.08	0.88	1.5	1.5	1.25	1.33	1.25	1.5	1.5	1.5
MEM + ETP	2	2	0.75	0.87	0.46[c]	0.36[c]	2	0.75	0.36[c]	0.36[c]	2	0.625	2
CAZ–AVI + ETP	0.5[c]	0.34[c]	0.14[c]	0.11[c]	0.18[c]	0.21[c]	0.16[c]	0.18[c]	0.14[c]	0.11[c]	0.5[c]	0.75	0.625
CAZ–AVI + IPM	0.25[c]	0.34[c]	0.17[c]	0.08[c]	0.17[c]	0.09[c]	0.21[c]	0.16[c]	0.25[c]	0.19[c]	0.25[c]	0.5[c]	0.375[c]
CAZ–AVI + MER	0.5[c]	0.5[c]	0.18[c]	0.18[c]	0.21[c]	0.21[c]	0.21[c]	0.18[c]	0.18[c]	0.18[c]	0.37[c]	0.5[c]	0.5[c]
CAZ–AVI + GEN	1	1.32	1.1	0.88	1	1	1	1	1.25	0.88	1.5	1.25	2
CAZ–AVI + TGC	1.31	1.32	1.5	1.25	1	0.75	1	1	0.67	0.50[c]	1.25	1.16	1.16
CAZ–AVI + CIP	1	1	1.6	1.41	1.25	1.5	1.25	1.5	1.5	1	1.5	2	2

CAZ, ceftazidime; CAZ–AVI, ceftazidime-avibactam; CIP, ciprofloxacin; CST, colistin; ESBL, extended-spectrum beta-lactamase; ETP, ertapenem; FIC, fractional inhibitory concentration; GEN, gentamicin; IPM, imipenem; MEM, meropenem; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; TGC, tigecycline.

[a] D179Y mutant bla_{KPC-3}.

[b] MIC for colistin was tested by broth microdilution method.

[c] Antimicrobial combinations with synergistic activity.

Table 2
Susceptible breakpoint index (SBPI) of antimicrobial combinations tested against ceftazidime–avibactam-susceptible and resistant KPC-producing *Klebsiella pneumoniae* isolates included in this study.

	CAZ–AVI-susceptible			CAZ–AVI-resistant
	Range	Median	Mean	Range
MEM + CAZ	0.06–0.08	0.06	0.07	0.069–0.08
MEM + ETP	0.07–0.41	0.18	0.20	0.07–0.09
CAZ–AVI + ETP	21.1–125.5	43.43	59.65	0.14–0.25
CAZ–AVI + IPM	32.3–125.5	64.5	64.69	0.5–31.41
CAZ–AVI + MEM	21.3–85.7	42.6	44.87	0.37–1.12
CAZ–AVI + GEN	10–22.38	18	16.08	1.03–1.39
CAZ–AVI + TGC	6.6–33.3	13.29	15.48	1.06
CAZ–AVI + CIP	3.2–21	10.67	10.71	0.03

CAZ, ceftazidime; CAZ–AVI, ceftazidime-avibactam; CIP, ciprofloxacin; ETP, ertapenem; GEN, gentamicin; IPM, imipenem; MEM, meropenem; TGC, tigecycline.

all KPC-Kp strains (Table 2). Analysis of the SBPI showed that combinations of double carbapenems and meropenem plus ceftazidime exhibited lower SBPI values against all KPC-Kp strains, while CAZ–AVI in combination with imipenem exhibited higher SBPI than other combinations against all CAZ–AVI-susceptible strains. At the same time, the combination of CAZ–AVI and imipenem exhibited a wide range of SBPI values (0.5–31.41) against CAZ–AVI-resistant KPC-Kp strains (Table 2).

Discussion

The study findings demonstrated that CAZ–AVI in combination with meropenem or imipenem displayed high synergistic activity against all KPC-Kp isolates, including CAZ–AVI-susceptible and resistant strains. Notably, the results also demonstrated that CAZ–AVI reduced the MICs for meropenem and imipenem below the resistance breakpoints for most KPC-3-producers. Additionally, CAZ–AVI in combination with imipenem exhibited higher SBPIs compared to other combinations against 12 of the 13 (92%) KPC-Kp strains, thus demonstrating a higher synergistic interaction between these antimicrobials in vivo. Indeed, the SBPI is a parameter that relates the magnitude of the interaction to the pharmacodynamic breakpoints used to determine susceptibility in vivo (Milne and Gould, 2010). At the same time, low SBPI values (<2) were observed for carbapenem/CAZ–AVI-resistant KPC-Kp strains, thus suggesting a weak synergistic interaction in vivo against this multidrug-resistant organism.

In these KPC-Kp strains, the resistance to CAZ–AVI was due to the D179Y mutation within the bla_{KPC-3} gene. Similar findings have been described in previous studies, which demonstrated specific mutations in bla_{KPC-3} to be associated with resistance to CAZ–AVI and to restored carbapenem susceptibility in different isolates (Shields et al. 2017; Haidar et al., 2017). At the same time, the in vitro synergistic activity and clinical efficacy of CAZ–AVI plus carbapenem combination has recently been reported in a patient with refractory bacteraemia due to a KPC-Kp strain (Camargo et al., 2015). Taken together, these findings and the results of the present study suggest that CAZ–AVI in combination with imipenem could represent a suitable option for infections due to KPC-Kp strains by restoring carbapenem activity. Further clinical studies will be essential to evaluate the clinical impact of this combination and establish the efficacy of this regimen in the treatment of infections due to KPC-Kp strains.

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Ethical approval

Not applicable.

Conflict of interest

REL has received research support from Merck and Gilead, and speaking fees from Merck, Gilead, and Basilea. All other authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2017.09.017.

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