MIC_{90} values obtained for macrolides (erythromycin, azithromycin, clarithromycin) also ranged from 0.5 to 1.0 mg/L. Interestingly, decreasing susceptibility to azithromycin was observed within the ST1 isolates in comparison with non-ST1 isolates. All ST1 isolates showed MICs from 0.25 to 1 mg/L while 96% of non-ST1 isolates did not have MICs >0.25 mg/L. These data correlate with the previously recognized relationship between STs and azithromycin susceptibility of clinical *Legionella* isolates.[8] It was found that rifampicin is the most active drug against isolates with an MIC_{90} of 0.032 mg/L under *in vitro* conditions. In contrast, doxycycline was found to be the least active drug, with an MIC_{90} of 2.0 mg/L.

In summary, the *in vitro* activity of the tested antibiotics against the German clinical isolates was in agreement with results from other studies.[8–10]

The introduction of routine susceptibility testing seems necessary for better understanding of the development of antibiotic resistance *in vivo* within the *L. pneumophila* population. The obtained MIC values can be used as a reference for defining ECOFFs and establishing the clinical breakpoints by EUCAST.

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Transparency declarations

None to declare.

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Ceftazidime/avibactam alone or in combination with aztreonam against colistin-resistant and carbapenemase-producing *Klebsiella pneumoniae*

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Sir,

The spread of carbapenemase-producing *Klebsiella pneumoniae* is a major public health concern since such isolates are basically resistant to most available antibiotics, including β-lactams, fluoroquinolones and aminoglycosides.[1] Infections due to carbapenemase-producing *K. pneumoniae* are therefore commonly treated with a regimen containing colistin.[1] However, acquired

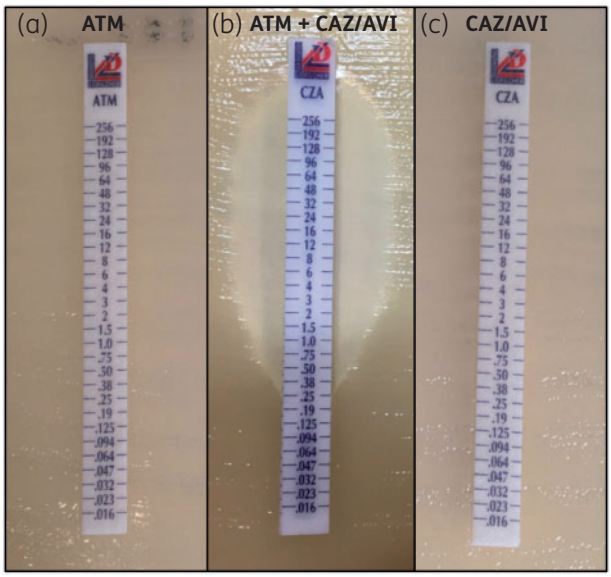

Figure 1. Example of synergistic combination of ceftazidime/avibactam (CAZ/AVI) and aztreonam (ATM) for an NDM + ESBL-producing *K. pneumoniae*. Susceptibility testing of ATM alone (a), combination of CAZ/AVI with ATM (b) and CAZ/AVI alone (c). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

resistance to colistin now occurs frequently and has few therapeutic options.[2] Outbreaks with colistin-resistant and carbapenemase-producing *K. pneumoniae* isolates have been reported worldwide[2] and mortality rates are high owing to limited treatment options.[3]

Recently, a new therapeutic option, namely ceftazidime/avibactam, combining a broad-spectrum cephalosporin and a novel β-lactamase inhibitor, has been marketed. The addition of avibactam expands the spectrum of activity of ceftazidime to many MDR Enterobacteriaceae including producers of ESBLs and carbapenemases.[4] Indeed, avibactam is active against all types of ESBLs and against carbapenemases of class A (KPC) and of some class D (OXA-48 and its derivatives), but is not active against class B β-lactamases (MBLs).[4]

In two reports, the combination of ceftazidime/avibactam with aztreonam demonstrated a synergistic effect against MBL-producing Gram-negative pathogens, but only a small number of isolates were tested.[5,6]

The objective of this study was to determine the *in vitro* activity of ceftazidime/avibactam, alone (for class A and D carbapenemase producers) or in combination with aztreonam (for class B carbapenemase producers), against a collection of colistin-resistant and carbapenemase-producing *K. pneumoniae* isolates.

A collection of 63 *K. pneumoniae* isolates recovered from clinical samples in France, Colombia and Turkey were tested in this study. All the isolates were resistant to colistin (MICs of colistin ranging from 8 to >128 mg/L) and produced a carbapenemase. The nature of the carbapenemase and the mechanisms responsible for colistin resistance have been previously characterized. Our collection included 11 KPC-like producers, 32 OXA-48 producers, 5 OXA-181 producers, 8 NDM-like producers and 7 isolates that co-produced two carbapenemases (NDM and OXA-48 or NDM and OXA-181) (Table S1, available as Supplementary data at *JAC* Online). The mechanisms responsible for colistin resistance were various and are indicated in Table S1.

MICs of ceftazidime/avibactam were determined using MIC test strips (Liofilchem, I2A, Montpellier, France) according to the manufacturer's guidelines. Following the EUCAST breakpoints (http://www.eucast.org/), isolates with an MIC of ceftazidime/avibactam of ≤8 mg/L were categorized as susceptible, whereas those with an MIC >8 mg/L were categorized as resistant. All the *K. pneumoniae* isolates producing a class A (KPC) or class D (OXA-48 and OXA-181) carbapenemase alone were susceptible to ceftazidime/avibactam with MICs ranging from 0.12 to 6 mg/L. As expected, the isolates producing a class B carbapenemase (NDM alone or associated with another carbapenemase) presented a high level of resistance to ceftazidime/avibactam (MIC ≥256 mg/L) (Table S1).

For the 15 isolates producing an MBL, MICs of aztreonam were also determined using MIC test strips (Liofilchem). According to EUCAST breakpoints, isolates with an MIC of ≤1 mg/L were categorized as susceptible, whereas those with an MIC >4 mg/L were categorized as resistant. Out of the 15 isolates, only a single isolate was actually susceptible to aztreonam. Resistance to aztreonam was mainly due to production of ESBLs (data not shown).

The *in vitro* synergy of ceftazidime/avibactam and aztreonam against MBL-positive isolates was studied using MIC test-strip-based synergy methods as previously described.[6] The combination was tested by first applying a ceftazidime/avibactam strip to the Mueller–Hinton agar, removing it after 5 min, then applying an aztreonam strip to the exact same location and replacing the ceftazidime/avibactam strip on top of the aztreonam strip.[6] Despite a high level of resistance to each antibiotic, the combination was synergistic for all the isolates with MICs of the combination <2 mg/L (Figure 1).

This study further suggests that ceftazidime/avibactam is an effective therapeutic option for treating infections caused by colistin-resistant and KPC- or OXA-48-producing *K. pneumoniae*. Moreover, the association of ceftazidime/avibactam and aztreonam is effective against NDM-producing *K. pneumoniae*. This combination was efficient in particular against *K. pneumoniae* isolates producing two carbapenemases. The synergy of the combination of ceftazidime/avibactam with aztreonam against NDM producers could be explained by the neutralization of the ESBL activity by avibactam allowing a restoration of the susceptibility to aztreonam. This study suggests that further commercialization of aztreonam/avibactam as a pharmaceutical preparation could be an interesting option to treat infections caused by MBL producers.

Notably, synergy of ceftazidime/avibactam and aztreonam can be easily evaluated using MIC test-strip synergy assays in clinical microbiology laboratories. Further investigations using experimental models and clinical trials are required to further confirm that this might be a relevant and effective therapeutic option in clinical practice.

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None to declare.

Supplementary data
Table S1 is available as Supplementary data at *JAC* Online.

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Emergence of multiple carbapenemase-producing organisms in single patients: an increasing threat to treatment of infection

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Sir,

The global spread of carbapenemase-producing organisms (CPOs) poses a growing threat to health services worldwide. The coexistence of various CPOs in single patients has been reported.[1–4] Recently, a study was conducted on a hospitalized patient from whom five CPOs were identified. The patient was a male in his 80s and he was admitted to the respiratory ICU (RICU) with lung and urinary tract infections in autumn 2014 and died 10 months later. While in the RICU he received multiple antibiotics, including piperacillin/tazobactam, ceftazidime, minocycline, moxifloxacin, meropenem and imipenem. This study did not require formal ethical approval, because we have only analysed the characteristics of clinical isolates that were collected

Table 1. Characteristics of carbapenemase-producing Gram-negative organisms from the same patient

Isolate no.	Species	Source	Isolation date (dd/mm/yyyy)	PFGE type	Carbapenemase(s)	MIC (mg/L)								
						CTX	CAZ	FEP	TZP	IPM	MEM	ETP	AMK	CIP
IRA001	*A. baumannii*	sputum	18/10/2014	ABA1	OXA-23, OXA-51	>64	>64	>64	>128	>16	>16	>8	>64	>4
IRP001	*P. aeruginosa*	sputum	05/11/2014	PAE1	VIM-1	>64	>64	>64	>128	>16	>16	>8	>64	>4
IR1247	*K. pneumoniae*	urine	04/02/2015	X	KPC-2, OXA-48	>64	>64	>64	>128	>16	>16	>8	>64	>4
IR5690	*K. pneumoniae*	urine	04/06/2015	A	OXA-48	>64	4	2	>128	2	2	>8	>64	>4
IR5691	*K. pneumoniae*	sputum	19/06/2015	A	OXA-48	>64	4	2	>128	2	2	>8	>64	>4
IR5692	*K. pneumoniae*	sputum	22/07/2015	A	OXA-48	>64	4	2	>128	2	2	>8	>64	>4
IR5693	*K. pneumoniae*	sputum	25/08/2015	A	OXA-48	>64	4	2	>128	2	2	>8	>64	>4
IR53017	*E. coli*	urine	01/07/2015	E	OXA-48	>64	16	>64	64	<1	<1	>8	>64	>4
IR5283	*E. cloacae*	sputum	21/08/2015	N/A	OXA-48	>64	4	16	64	8	4	4	>64	>4

CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin/tazobactam; IPM, imipenem; MEM, meropenem; ETP, ertapenem; AMK, amikacin; CIP, ciprofloxacin; N/A, not applicable.