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Short Communication

Activity of aztreonam/avibactam and ceftazidime/avibactam against Enterobacterales with carbapenemase-independent carbapenem resistance

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ABSTRACT

Enterobacterales with carbapenemase-independent resistance to carbapenems are sometimes selected during therapy and, on rare occasions, cause outbreaks. Most have extended-spectrum or AmpC β-lactamases, together with changes to permeability or penicillin-binding proteins (PBPs). Newer β-lactam–β-lactamase inhibitor combinations may present useful options for infections due to these organisms. Accordingly, Clinical and Laboratory Standards Institute/European Committee on Antimicrobial Susceptibility Testing broth-microdilution was used to measure the minimum inhibitory concentrations (MICs) of ceftazidime/avibactam and aztreonam/avibactam for 51 carbapenemase-negative Enterobacterales with resistance or reduced susceptibility to carbapenems: genomic sequencing of the least-susceptible organisms was also undertaken. MICs of the two avibactam combinations cross-correlated closely, but with fewer MICs (2/51 vs. 10/51) exceeding 8+4 mg/L in the case of ceftazidime/avibactam. Raised MICs for *Escherichia coli* were associated with PBP3 inserts together with CMY-42 β-lactamase; correlates among *Enterobacter cloacae* complex isolates remain elusive, with AmpC and PBP3 sequences found to be species specific. In the case of *Klebsiella* spp., no MICs exceeding 2 mg/L were seen for either combination. It appears that these avibactam combinations have potential against Enterobacterales with carbapenemase-independent carbapenem resistance or reduced susceptibility, with ceftazidime/avibactam being more reliably active than aztreonam/avibactam.

Key findings

- Minimum inhibitory concentrations (MICs) of aztreonam/avibactam and ceftazidime/avibactam correlated closely for Enterobacterales with carbapenemase-independent carbapenem resistance.
- At a breakpoint of 8+4 mg/L, ceftazidime/avibactam was the more reliably active of the two avibactam combinations
- Raised avibactam combination MICs for *Escherichia coli* were associated with penicillin-binding protein (PBP)3 inserts and CMY-42 β-lactamase.
- Correlates of raised MICs for *Enterobacter cloacae* complex isolates remain unclear, with PBP3 and AmpC sequences being species related.

1. Introduction

Carbapenemase-independent resistance to carbapenems receives less attention than the spread of carbapenemases. This is because carbapenemases are often plasmid-mediated and able to spread among bacteria, whereas carbapenemase-independent resistance is mutational and cannot spread [1]. Moreover, carbapenemases generally confer higher-level resistance, particularly in the cases of metallo- and KPC enzymes.

Nevertheless, carbapenemase-independent resistance should not be dismissed. It is sometimes selected during therapy, precipitating treatment failure [2,3], typically when mutations or in-

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dels interrupt porin genes in strains already producing AmpC or extended-spectrum β-lactamase (ESBL) enzymes. The widespread view that such mutants are rendered unfit by their porin deficiencies is challenged by the fact that they occasionally cause outbreaks, affecting multiple patients [4,5].

Previously, the present authors showed that ceftazidime/avibactam remains active at its 8+4 mg/L European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) breakpoint against almost all Enterobacterales with carbapenemase-independent resistance to ertapenem [6]. The present study extends these investigations, comparing the behaviours of ceftazidime/avibactam and aztreonam/avibactam, and examining the mechanisms present in the least-susceptible isolates.

2. Materials and methods

In total, 51 isolates were tested, comprising 20 *Escherichia coli*, 19 *Klebsiella* spp. (18 *K. pneumoniae* and one *K. aerogenes*) and 12 *Enterobacter cloacae* complex organisms. All had been referred to the UK Health Security Agency (UKHSA) Antimicrobial Resistance and Healthcare Associated Infections Reference Unit between 2015 and 2019, and were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker BioTyper, Bruker Daltonics, Bremen, Germany). Minimum inhibitory concentrations (MICs) of ertapenem, as determined by British Society for Antimicrobial Chemotherapy agar dilution, were ≥8 mg/L for 50 of 51 isolates and, for all except one, were ≥4 mg/L for either or both of imipenem and meropenem. Despite these raised MICs of carbapenems, all 51 isolates were carbapenemase-negative based upon polymerase chain reaction for bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA\text{-}48\text{-}like}$, bla_{KPC}, bla_{GES} and bla_{FRI}, and modified carbapenem-inactivation-method tests.

MICs of aztreonam, aztreonam/avibactam, ceftazidime and ceftazidime/avibactam were determined by broth-microdilution in cation-adjusted Mueller–Hinton media, following EUCAST/CLSI methodology [7,8]. Amikacin, ciprofloxacin, colistin, meropenem and tigecycline were tested in parallel on the same broth-microdilution plates. Test plates were sourced from International Health Management Associates (Schaumburg, IL, USA). Avibactam was used at 4 mg/L. Full details of the MIC testing are given elsewhere [9].

Whole genome sequencing (WGS) was undertaken, using Illumina methodology, for isolates with aztreonam/avibactam or ceftazidime/avibactam MICs ≥8 mg/L (i.e. at or above the prospective breakpoint). Details are given elsewhere [9]. WGS data for *E. cloacae* complex isolates were related to clustering that had been generated previously during studies on colistin resistance [10]. Phylogenetic trees were constructed using a maximum likelihood statistical method (RAxML) [11,12] based on: (i) whole Enterobacter genomes; and (ii) the sequences of *ampC* and *ftsI*, encoding the chromosomal AmpC β-lactamase and penicillin-binding protein (PBP)3, respectively.

3. Results

3.1. Antimicrobial susceptibility testing

The isolates were selected based on resistance to ertapenem, and resistance or reduced susceptibility to meropenem in prior testing by agar dilution. In the present broth-microdilution testing, 10 of 51 isolates proved resistant to meropenem at EUCAST's MIC >8 mg/L breakpoint, and 33 of 51 isolates proved resistant to meropenem at CLSI's MIC >2 mg/L breakpoint (Table 1); meropenem MICs for all of the isolates were above the EUCAST's ECOFF values of 0.06 mg/L for *E. coli*, 0.12 mg/L for *K. pneumoniae* and 0.25 mg/L *for E. cloacae*. Ertapenem was not retested. All the isolates were unequivocally resistant to aztreonam and ceftazidime in the absence of a β-lactamase inhibitor, with MICs >16 mg/L. Over 80% remained susceptible to colistin 2 mg/L and amikacin 8 mg/L, corresponding to EUCAST breakpoints. Tigecycline appeared widely active if the US Food and Drug Administration criteria (susceptible, MIC ≤2 mg/L) were adopted; EUCAST only has a breakpoint (0.5 mg/L) for *E. coli*. Ciprofloxacin resistance was widespread, with 31 of 51 MICs exceeding EUCAST's 0.5 mg/L breakpoint.

MICs of aztreonam/avibactam exceeded 8+4 mg/L (the prospective breakpoint) for 10 of 51 isolates, comprising seven of 20 *E. coli* and three of 12 *E. cloacae* complex. In the case of ceftazidime/avibactam, only two *E. coli* isolates were resistant at the established CLSI and EUCAST breakpoint of 8+4 mg/L. Aztreonam/avibactam MICs for a further three *E. cloacae* complex isolates were 8+4 mg/L. All 19 *Klebsiella* spp. were inhibited by both of the avibactam combinations at ≤2 mg/L.

Table 2 provides a cross-plot of aztreonam/avibactam vs. ceftazidime/avibactam MICs. Except for a single *Enterobacter* sp. isolate (indicated, see footnote), MICs of both combinations were strongly inter-related, although those for aztreonam/avibactam were distributed more widely.

3.2. Investigation of isolates with raised MICs to avibactam combinations

WGS was undertaken for the seven *E. coli* and six *E. cloacae* complex isolates for which aztreonam/avibactam MICs were ≥8 mg/L. Six of the seven *E. coli* had inserts in *ftsI*. These indicated insertions of Tyr-Arg-Ile-Asn (YRIN) after Pro 333 of PBP3 in three cases, and Tyr-Arg-Ile-Lys (YRIK) in a further three cases (Table 3). All six also carried acquired AmpC enzymes – CMY-42 in five cases and CMY-146 in one case – together with variable combinations of TEM, CTX-M and OXA-1 β-lactamases (Table 3). The three *E. coli* with YRIN all belonged to ST361: two were from a single site and possessed CMY-42 β-lactamase, but were isolated 5 months apart and differed in respect of their secondary β-lactamases, indicating that they were not replicates. All three *E. coli* with YRIK inserts had CMY-42 enzymes and belonged to the well-known ST405 (n=1) and ST410 (n=2) high-risk clones; all three were from different sites, but the two ST410 isolates had the same secondary β-lactamases and may represent members of a single lineage. No PBP3 inserts, nor other obvious mechanisms of resistance, were present in the remaining 'resistant' *E. coli*, an ST131 isolate with CTX-M-15 and OXA-1 β-lactamases (ESCOL30, Table 3); resistance in this isolate could not be confirmed in retesting by gradient strip, and its mechanism seemingly had been lost.

Sequences of *ftsI* varied among the six *E. cloacae* complex isolates with aztreonam/avibactam MICs ≥8 mg/L. However, there was no evidence of YRIN or related inserts to PBP3 and, whilst some observed variation may relate to reduced susceptibility to avibactam combinations, it is likely that much reflects endogenous diversity within the species complex. WGS indicated that the six enterobacters variously belonged to our previously-defined [10] genomic clusters F (n=3), A (n=2) and C (n=1), which correspond to *E. xiangfangensis, E. kobei* and *E. asburiae*, respectively, in the updated *E. cloacae* complex taxonomy (Fig. S1a, see online supplementary material). Phylogenetic trees constructed solely on *ampC* and *ftsI* (Fig. S1b,c, respectively, see online supplementary material) mirrored those based on whole genome data, supporting the view that these genes have substantial species specificity within the *E. cloacae* complex. Given the tiny numbers of resistant isolates per species cluster, it was not possible to convincingly associate microvariation in *ampC* or *ftsI* with raised MICs for avibactam combinations.

Table 1
Minimum inhibitory concentration (MIC) distributions for the test panel, by genus.

	No. isolates with indicated MIC, mg/L														
Aztreonam	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	Total
E. coli											2	2	2	14	20
Klebsiella spp.												3	2	14	19
E. cloacae complex											1		5	6	12
All isolates											3	5	9	34	51
Aztreonam/avibactam															
E. coli		1	1	4	5	1	1			3	3	1			20
Klebsiella spp.			2	5	7	3	2								19
E. cloacae complex				1	2	1	1	1	3	2	1				12
All isolates	2	1	3	10	14	5	4	1	3	5	4	1			51
Ceftazidime															
E. coli										1		3	3	13	20
Klebsiella spp.											3	2	3	11	19
E. cloacae complex											1		3	8	12
All isolates										1	4	5	9	32	51
Ceftazidime/avibactam															
E. coli				1	7	3	2	2	3	2					20
Klebsiella spp.					3	9	6	1							19
E. cloacae complex					2	2	5	2	1						12
All isolates				1	12	14	13	5	4	2					51
Meropenem															
E. coli						1	9	3	4	3					20
Klebsiella spp.					1		3	5	6	4					19
E. cloacae complex						2	2	3	2	1	1	1			12
All isolates					1	3	14	11	12	8	1	1			51
Amikacin															
E. coli						4	4	6		5		1			20
Klebsiella spp.					5	4	1	4	1		2		2[a]		19
E. cloacae complex					3	8			1						12
All isolates					8	16	5	10	2	5	2	1	2[a]		51
Ciprofloxacin															
E. coli											1	3	16[a]		20
Klebsiella spp.		1	2	3	2			1	1		2	2	5[a]		19
E. cloacae complex	10	1	1												12
All isolates	10	2	3	3	2			1	1		3	5	21		51
Colistin															
E. coli				17[b]	2	1									20
Klebsiella spp.				6[b]	5	1	1	1	5[a]						19
E. cloacae complex				9[b]	1				2[a]						12
All isolates				32[b]	8	2	1	1	7[a]						51
Tigecycline															
E. coli		1	10	7	2										20
Klebsiella spp.		1		2	5	5	1	4	1						19
E. cloacae complex				1	9	2									12
All isolates		**2**	**10**	**10**	**16**	**7**	**1**	**4**	**1**						**51**

E. coli, Escherichia coli; E. cloacae, Enterobacter cloacae complex.
[a] MIC > indicated value.
[b] MIC ≤ indicated value.

Table 2
Cross-plot of aztreonam/avibactam vs. ceftazidime/avibactam minimum inhibitory concentrations (MICs) for all 51 isolates.

MIC ceftazidime/ avibactam, (mg/L)	No. isolates with indicated MIC of aztreonam/avibactam (mg/L)												
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	Total
0.25					1								1
0.5		1	1	5	3	1					1[a]		12
1			2	4	5	1	2						14
2				1	5	3	1	1	2				13
4							1		1	3			5
8										2	2		4
16											1	1	2
Grand total	0	1	3	10	14	5	4	1	3	5	4	1	51

[a]Exceptional isolate with resistance, or relative resistance, to only one or other avibactam combination. Grey boxes indicate the line of equivalence.

Table 3
Penicillin-binding protein (PBP)3 (*ftsI* gene) changes and β-lactamases among seven *Escherichia coli* isolates with aztreonam/avibactam minimum inhibitory concentrations (MICs) ≥8 mg/L

Isolate	PBP3 insert	MLST	β-lactamases	MIC (mg/L)							
				Aztreo-nam	Aztreonam/ avibactam	Mero-penem	Ceftaz-idime	Ceftazidime/ avibactam	Amikacin	Cipro-floxacin	Colistin	Tige-cycline
ESCOL7	YRIN	361	TEM-1B, **CMY-42**	64	16	2	>128	4	1	>64	0.5	0.25
ESCOL8	YRIN	361	CTX-M-15, OXA-1, **CMY-42**	>128	16	2	>128	4	16	>64	≤0.25	0.125
ESCOL13	YRIK	410	OXA-1, CTX-M-15, **CMY-42**	>128	32	4	>128	8	2	>64	2	0.125
ESCOL17	YRIK	410	CTX-M-15, OXA-1, **CMY-42**	>128	16	2	>128	8	16	>64	0.5	0.25
ESCOL18	YRIK	405	TEM-35, TEM-169 **CMY-42**	128	64	2	>128	16	16	>64	≤0.25	0.25
ESCOL20	YRIN	361	TEM-1C, CMY-146	>128	32	16	>128	16	1	>64	≤0.25	0.5
ESCOL30	None	131	CTX-M-15, OXA-1	>128	32	16	>128	8	4	32	≤0.25	0.125

MLST, multi-locus sequence type, as deduced from WGS data.

4. Discussion

These data illustrate the wide activity of both aztreonam/avibactam and ceftazidime/avibactam against Enterobacterales with carbapenemase-independent carbapenem resistance. Nonetheless, MICs of the avibactam combinations for these isolates were notably higher than the values of ≤1 mg/L seen for the great majority of ESBL- and AmpC-producing Enterobacterales [13,14].

MICs of both avibactam combinations were inter-related (Table 2); however, MICs were more widely distributed for aztreonam/avibactam than for ceftazidime/avibactam. This appears to reflect two factors: first, aztreonam typically has lower MICs than ceftazidime for fully-susceptible Enterobacterales; and second, aztreonam is more vulnerable to compromise via target (PBP3) modification, probably because it solely targets the D-Ala-D-Ala transpeptidase activity of this protein, whereas ceftazidime also attacks PBP1a and 1b [15,16]. PBP3 inserts known to reduce antibiotic binding were found in six of seven *E. coli* isolates with aztreonam/avibactam MICs ≥8 mg/L with either YRIN or YRIK inserted after Pro333. Five of the six also carried $bla_{\text{CMY-42}}$, encoding an acquired AmpC variant with increased activity against ceftazidime and aztreonam [17]; the sixth carried $bla_{\text{CMY-146}}$, encoding a less-studied acquired AmpC variant. For comparison, in recent studies [9,18 and unpublished], the authors have sequenced a total of 48 *E. coli* isolates that were susceptible to aztreonam with or without avibactam at ≤0.5 mg/L. Actual MICs ranged from <0.015 mg/L to 0.5 mg/L, with a single mode at 0.12 mg/L. CMY-42 β-lactamase was not found in any of these isolates; YRIN inserts were present in just six of 48 isolates, four of them with MICs of 0.5 mg/L (i.e. at the upper edge of the distribution).

Although PBP3 inserts have mainly been studied in metallo-β-lactamase-producing Enterobacterales, particularly those with NDM enzymes, it is clear from the present data, and from the reports of others [19], that they are more widespread. Indeed, it is highly likely that the common pathway of events is for isolates with pre-existing PBP3 modification to acquire plasmids encoding metallo-β-lactamases, rather than for metallo-β-lactamase producers to develop PBP3 modifications *de novo* [20]. Notably, the three resistant *E. coli* isolates with YRIN inserts all belonged to ST361, and the three isolates with YRIK inserts variously belonged to ST405 and ST410. All three sequence types – with the same PBP3 inserts – were represented among aztreonam/avibactam-resistant *E. coli* with NDM carbapenemases collected in the UK during the same period; ST405 isolates with YRIK inserts together with NDM-5 enzymes are particularly widespread [9].

The situation with *E. cloacae* is more uncertain than for *E. coli*. Variation in *ftsI* was seen, but with no clear correlate of raised MICs for the avibactam combinations. Moreover, and critically, '*E. cloacae*' is a complex rather than a single species and both *ampC* and *ftsI* sequences had a degree of species specificity. Resolving these issues will require studies with larger collections.

In summary, these data illustrate the wide activity of both avibactam combinations against Enterobacterales with carbapenemase-independent resistance to carbapenems. The occurrence of resistance and reduced susceptibility in *E. coli* with combinations of PBP3 inserts and CMY-42 β-lactamases is, nonetheless, concerning, as are raised MICs for some *E. cloacae* complex isolates. Ceftazidime/avibactam was less affected than aztreonam/avibactam by these mechanisms and, based on the present evidence, appears to be the preferable option for infections due to such strains.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2023.107081.

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