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Aztreonam/avibactam activity against a large collection of carbapenem-resistant Enterobacterales (CRE) collected in hospitals from Europe, Asia and Latin America (2019–21)

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Background: Aztreonam/avibactam is under development to treat infections caused by Gram-negative bacteria. We evaluated the *in vitro* activities of aztreonam/avibactam and comparators against a global collection of carbapenem-resistant Enterobacterales (CRE), including ceftazidime/avibactam-resistant isolates.

Methods: Isolates were consecutively collected (24 924; 1/patient) from 69 medical centres in 36 countries during 2019–21. Isolates were susceptibility tested by CLSI broth microdilution. All CRE isolates ($n = 1098$; 4.4%) were *in silico* screened for carbapenemase (CPE) genes after genome sequencing. CRE susceptibility results were stratified by CPE, geography and resistance phenotype.

Results: Aztreonam/avibactam inhibited 99.6% of CREs at ≤8 mg/L ($MIC_{50/90}$, 0.25/0.5 mg/L), including 98.9% (345/349) of ceftazidime/avibactam-resistant isolates. Aztreonam/avibactam activity was consistent across geographical regions (98.9%–100.0% inhibited at ≤8 mg/L), but susceptibility to comparators varied markedly. Susceptibility (CLSI criteria) for ceftazidime/avibactam and meropenem/vaborbactam ranged from 80.2% and 77.5% in Western Europe to 39.5% and 40.3% in the Asia-Pacific region, respectively. Aztreonam/avibactam retained activity against isolates non-susceptible to colistin (99.7% inhibited at ≤8 mg/L) or tigecycline (98.6% inhibited at ≤8 mg/L). A CPE gene was identified in 972 CRE isolates (88.5%). The most common CPEs were KPC (43.1% of CREs), NDM (26.6%) and OXA-48–like (18.7%); 57 isolates (5.2%) had >1 CPE gene. Aztreonam/avibactam inhibited 99.9% of CPE producers at ≤8 mg/L, whereas ceftazidime/avibactam and meropenem/vaborbactam exhibited limited activity against isolates producing MBL and/or OXA-48-like enzymes.

Conclusions: Aztreonam/avibactam activity was not adversely affected by clinically relevant CPEs. Our results support aztreonam/avibactam development to treat infections caused by CRE, including MBL producers.

Introduction

Among most Enterobacterales species, resistance to carbapenems is primarily due to the production of carbapenemases. For therapeutic purposes, the carbapenemases produced by Enterobacterales can be divided in two groups, the serine carbapenemases, such as KPCs and oxacillinase-48 (OXA-48), and the MBLs.[1]

The main strategy to counteract carbapenem resistance in the Enterobacterales has been the development of β-lactamase inhibitors (BLIs). Many BLIs have been approved recently by the US FDA to be used in combination with various β-lactams (BLs). Some of these new BL/BLI combinations, particularly ceftazidime/avibactam, meropenem/vaborbactam and imipenem/relebactam, have shown potent *in vitro* activity and clinical efficacy against carbapenem-resistant Enterobacterales (CRE) producing class A carbapenemase; however, the current clinically available BL/BLI combinations are not active against MBL-producing Enterobacterales.[2]

MBLs are of particular concern due to their ability to hydrolyse virtually all β-lactams, their rapid development of new variants, the horizontal transferability of their encoding genes and, most importantly, the lack of clinically useful MBL inhibitors.[1,3] One strategy to overcome MBL-derived resistance is to combine an MBL-stable β-lactam with a serine carbapenemase inhibitor. A serine inhibitor would protect the MBL-stable β-lactam against ESBLs, AmpC β-lactamases and/or serine carbapenemases, often present in MBL-producing Enterobacterales.[4] Thus, the

combination of aztreonam with avibactam has showed great potential for successfully treating infections caused by Enterobacterales by broadening the spectrum of this combination against most classes of β-lactamases, including MBLs.[5]

Aztreonam was approved by the US FDA and the EMA in 1986, and remains the only clinically available member of the monobactam class.[6] Aztreonam is stable to hydrolysis by MBLs, but it is hydrolysed by most clinically relevant β-lactamases, such as ESBLs, AmpCs and KPCs. Avibactam is a non-β-lactam BLI that inhibits most clinically relevant serine β-lactamases and its clinical utility has been demonstrated by the efficacy of ceftazidime/avibactam in the treatment of infections caused by serine carbapenemase-producing CRE.[7] In the present study, we evaluated the *in vitro* activities of aztreonam/avibactam and comparators against a global (ex-USA) collection of CRE, including ceftazidime/avibactam-resistant isolates. We also evaluated the resistance mechanisms associated with decreased susceptibility to aztreonam/avibactam.

Materials and methods

Organism collection

Bacterial isolates were collected via a network of medical sites participating in the SENTRY Antimicrobial Surveillance Program, and sent to JMI Laboratories (North Liberty, IA, USA) for susceptibility testing.[8] Each participating centre was asked to collect consecutive bacterial isolates from patients hospitalized with the following infection types: bloodstream infection, pneumonia, skin and skin structure infection, urinary tract infection and intra-abdominal infection. The number of isolates per infection type and portion of the year varied slightly by region. Isolates could be from any specimen type associated with the infections above, if determined to be significant by local criteria as the reported probable cause of infection.

A total of 24 924 Enterobacterales isolates were collected consecutively during 2019–21 from 69 medical centres located in Western Europe [W-EU; $n=13\,125$; 26 centres in 10 countries (1 in Belgium, 4 in France, 6 in Germany, 1 in Ireland, 4 in Italy, 1 in Portugal, 3 in Spain, 2 in Sweden, 1 in Switzerland and 3 in the UK)], Eastern Europe and the Mediterranean [E-EU; $n=4404$; 15 centres in 10 countries (1 in Belarus, 1 in Czech Republic, 1 in Greece, 1 in Hungary, 2 in Israel, 1 in Poland, 1 in Romania, 3 in Russia, 2 in Slovenia and 2 in Turkey)], Asia-Pacific [APAC; $n=4270$; 17 centres in 9 countries (5 in Australia, 4 in Japan, 1 in Malaysia, 1 in New Zealand, 1 in Philippines, 2 in South Korea, 1 in Taiwan, 1 in Thailand and 1 in Vietnam) and Latin America (LATAM; $n=3035$; 11 centres in 7 countries (2 in Argentina, 3 in Brazil, 1 in Chile, 1 in Colombia, 1 in Costa Rica, 2 in Mexico and 1 in Panama]). Species identification was confirmed by using standard biochemical tests and/or a MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA) and/or genome sequencing, when necessary.

Susceptibility testing

All isolates were susceptibility tested by the reference broth microdilution method specified by CLSI standards. Aztreonam/avibactam was tested with avibactam at a fixed concentration of 4 mg/L. All tests were conducted in a central monitoring laboratory (JMI Laboratories). CRE was defined as displaying imipenem or meropenem MIC values of ≥4 mg/L. Imipenem was not applied to *Proteus mirabilis* or indole-positive Proteeae due to their intrinsically elevated MIC values. Overall, 1098 (4.4%) CRE isolates were identified for further molecular evaluation.[9]

A tentative aztreonam/avibactam pharmacokinetic/pharmacodynamic (PK/PD) susceptible breakpoint of ≤8 mg/L was applied for comparison.[10] CLSI and EUCAST breakpoints were applied to the comparator agents where available.[11,12] US FDA breakpoints were applied for tigecycline, whereas tigecycline breakpoints published by EUCAST for *Escherichia coli* and *Citrobacter koseri* (≤0.5 mg/L) were applied to all Enterobacterales species for comparison. Concurrent quality control (QC) testing was performed to ensure proper test conditions and procedures.

β-Lactamase screening and molecular characterization of isolates with decreased susceptibility to aztreonam/avibactam

All CRE isolates ($n=1098$) were genome sequenced for *in silico* screening of β-lactamase-encoding genes. Briefly, total genomic DNA was extracted using the fully automated ThermoScientific™ KingFisher™ Flex Magnetic Particle Processor (Cleveland, Ohio, USA). Libraries were normalized using the bead-based normalization procedure (Illumina) and sequenced on MiSeq. FASTQ format files for each sample set were assembled independently using the *de novo* assembler SPAdes 3.15.3 with K-values of 21, 33, 55, 77 and 99 plus careful mode on to reduce the number of mismatches. This process produced FASTA-format files of contiguous sequences with the best N50 value. An in-house proprietary bioinformatic pipeline and a JMI-curated resistance gene database (Version 3; uses Python v2.7.9, SPAdes v3.15.3 and BBMap v36.x) based on the NCBI Bacterial Antimicrobial Resistance Reference Gene Database (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047) was used for the *in silico* analysis. This resistance gene database was used as queries to align β-lactamase resistance determinants against the target assembled sequences. Hits with identities greater than 94% and 40% minimum coverage length were selected for further analysis and final assignment of β-lactamase alleles.[13,14]

Expression of *acrA* and *ampC* was performed by extracting total mRNA from cultures grown to mid-log phase in tryptic soy broth at 37°C with shaking followed by purification using the RNeasy Mini Kit in the Qiacube (QIAGEN) workstation according to the manufacturer's instructions. Residual DNA was eliminated by treatment with RNAse-free DNase (Promega, Madison, WI, USA). Quantification of mRNA and sample quality were assessed using the RNA 6000 Nano kit on the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. The relative transcription levels of *acrA* and *ampC* were determined by real-time PCR assays in the StepOne Plus instrument (Life Technologies, Foster City, CA, USA) using the Power SYBR Green RNA-to-C_T 1-Step qPCR master mix (Applied Biosystems, ThermoFisher) with 0.5 ng input RNA. Assays were performed in triplicate. Transcription levels of target genes were measured by the quantification of the target gene mRNA using a normalized expression analysis method relative to a housekeeping reference gene (*rpsL* in *E. coli* and *Enterobacter cloacae* species complex and *gyrA* in *Klebsiella pneumoniae*) compared with a susceptible control strain.[15,16] Oligonucleotides used in this study are listed in Table S1, available as Supplementary data at *JAC-AMR* Online.

Results

K. pneumoniae ($n=878$) accounted for 80.0% of CRE isolates. The second most common CRE species/group was *E. cloacae* species complex ($n=65$; 5.9%), followed by *E. coli* ($n=58$; 5.3%) and *Serratia marcescens* ($n=36$; 3.3%). The CRE isolates were collected from patients with bloodstream infections ($n=326$; 29.7% of total), pneumonia ($n=321$; 29.2%), urinary tract infections ($n=191$; 17.4%), skin and soft tissue infections ($n=152$; 13.8%), intra-abdominal infections ($n=86$; 7.8%) and other infection types ($n=22$; 2.0%). CRE rates were highest in E-EU

Table 1. Aztreonam/avibactam MIC distributions

	No. of isolates and cumulative % inhibited at aztreonam/avibactam MIC (mg/L) of:												
Organism group (*n*)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	MIC_{50}	MIC_{90}
CRE (1098)	74	118	243	394	189	44	23	8	1	1	3	0.25	0.5
	6.7	17.5	39.6	75.5	92.7	96.7	98.8	99.5	99.6	99.7	100.0		
KPC producers (473)	14	50	111	200	78	16	3	0	0	1		0.25	0.5
	3.0	13.5	37.0	79.3	95.8	99.2	99.8	99.8	99.8	100.0			
MBL producers (347)	59	59	86	88	32	12	7	4				0.12	0.5
	17.0	34.0	58.8	84.1	93.4	96.8	98.8	100.0					
OXA-48-like producers (205)	2	12	43	85	52	7	1	2	1			0.25	0.5
	1.0	6.8	27.8	69.3	94.6	98.0	98.5	99.5	100.0				
≥2 carbapenemases (57)	3	5	13	23	10	1	0	2				0.25	0.5
	5.3	14.0	36.8	77.2	94.7	96.5	96.5	100.0					
No carbapenemase (126)	1	1	15	42	38	10	12	4	0	0	3	0.5	2
	0.8	1.6	13.5	46.8	77.0	84.9	94.4	97.6	97.6	97.6	100.0		
CZA-R (349)	53	59	86	91	33	12	7	4	0	1	3	0.12	0.5
	15.2	32.1	56.7	82.8	92.3	95.7	97.7	98.9	98.9	99.1	100.0		
Colistin-R (292)	17	12	61	124	63	11	2	1	0	1		0.25	0.5
	5.8	9.9	30.8	73.3	94.9	98.6	99.3	99.7	99.7	100.0			
Tigecycline-NS (515)[a]	27	34	116	202	101	27	5	1	0	0	2	0.25	0.5
	5.2	11.8	34.4	73.6	93.2	98.4	99.4	99.6	99.6	99.6	100.0		

CZA, ceftazidime/avibactam; R, resistant; NS, non-susceptible.

[a]Isolates with tigecycline MIC values >0.5 mg/L, which is the susceptible breakpoint published by EUCAST for *E. coli* and *C. koseri*.

(454/4404; 10.3%), followed by LATAM (240/3035; 7.9%), APAC (177/4270; 4.1%) and W-EU (227/13 125; 1.7%).

Aztreonam/avibactam ($MIC_{50/90}$, 0.25/0.5 mg/L) inhibited 99.6% of CRE isolates (1094/1098) at ≤8 mg/L (96.7% at ≤1 mg/L), including all (100.0%) isolates producing MBLs or OXA-48-like, 99.8% (472/473) of KPC producers and 97.6% (123/126) of non-carbapenemase-producing CRE (Table 1). Aztreonam/avibactam was also highly active against CRE isolates resistant to ceftazidime/avibactam (*n* = 349; 98.9% inhibited at ≤8 mg/L), colistin (colistin MIC, >2 mg/L; *n* = 292; 99.7% inhibited at ≤8 mg/L) or tigecycline (tigecycline MIC, >0.5 mg/L; *n* = 515; 99.6% inhibited at ≤8 mg/L; Table 1). Notably, an MBL was identified in 97.4% (340/349) of ceftazidime/avibactam-resistant isolates (data not shown).

The activities of aztreonam/avibactam and comparator agents against the CRE collection and the four most common Enterobacterales species are shown in Table 2. The most active comparator agents were ceftazidime/avibactam ($MIC_{50/90}$, 2/>32 mg/L; 68.2% susceptible per CLSI and EUCAST), meropenem/vaborbactam ($MIC_{50/90}$, 2/>32 mg/L; 60.5%/64.3% susceptible per CLSI/EUCAST), amikacin ($MIC_{50/90}$, 8/>32 mg/L; 64.0%/53.7% susceptible per CLSI/EUCAST), minocycline ($MIC_{50/90}$, 4/>32 mg/L; 57.8% susceptible per CLSI), tigecycline ($MIC_{50/90}$, 0.5/2 mg/L; 93.4%/53.1% susceptible per US FDA/EUCAST) and colistin ($MIC_{50/90}$, 0.25/>8 mg/L; 73.3% susceptible per EUCAST; Table 2). When comparing susceptibility rates for isolates collected in 2019 with those collected in 2021, we observed that susceptibility rates improved slightly for ceftazidime/avibactam [from 63.7% to 68.6% (CLSI and EUCAST)] and meropenem/vaborbactam [from 55.8%/59.6% to 59.9%/64.0% (CLSI/EUCAST)], decreased slightly for amikacin [from 63.0%/51.6% to 60.3%/50.7% (CLSI/EUCAST)] and decreased markedly for colistin [from 80.0% to 67.8% (EUCAST); data not shown]. Moreover, *K. pneumoniae* represented 69.2%, 82.4% and 85.5% of isolates resistant to ceftazidime/avibactam, meropenem/vaborbactam and amikacin, respectively, and 80.8% of isolates non-susceptible to colistin per CLSI criteria (data not shown).

Although the main mechanism of carbapenem resistance in all four geographical regions was the production of a carbapenemase, carbapenemase genes varied substantially by region, as shown in Table 3. Genes encoding KPC predominated in W-EU (66.5% of CRE) and LATAM (70.0% of CRE). KPC-3 predominated in W-EU (86.8% of KPCs) and KPC-2 predominated in LATAM (94.0% of KPCs). MBL genes were detected in 61.6% of CRE isolates from the APAC region; NDM represented 92.7% (101/109) of these MBLs. A variety of carbapenemase genes were observed among E-EU countries. Overall, KPC, MBL and OXA-48-like represented 25.6%, 29.5% and 31.7% of CRE isolates from E-EU, respectively. KPC predominated in Greece and Romania, while OXA-48-like predominated in Russia and Turkey. MBLs, mainly NDM, were common in Belarus, Greece, Poland, Russia and Turkey.

Notably, combinations of two carbapenemase genes were identified in 57 (5.2%) isolates and three carbapenemase genes were identified in 1 (0.1%) isolate (Table 3). Isolates with two carbapenemase genes were mainly *K. pneumoniae* (84.2%; 48/57) and were isolated in E-EU (*n* = 23; 40.4%), APAC (*n* = 20; 35.0%), W-EU (*n* = 12; 21.1%) and LATAM (*n* = 2; 3.5%). The countries with the highest percentages of CRE isolates harbouring two carbapenemases genes were Thailand (38.2%; 13/34), Vietnam

Table 2. Activity of aztreonam/avibactam and comparator antimicrobial agents tested against all 1098 CRE isolates combined and the four most common species (2019–21)

	mg/L			CLSI/US FDA[a]			EUCAST[a]		
Antimicrobial agent (no. of isolates)	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R	%S	%I	%R
All Enterobacterales (1098)									
Aztreonam/avibactam[b]	0.25	0.5	≤0.03 to >16	(99.6)[b]			(99.6)[b]		
Ceftazidime/avibactam	2	>32	≤0.015 to >32	68.2		31.8	68.2		31.8
Meropenem/vaborbactam	2	>32	≤0.015 to >32	60.5	3.8	35.7	64.3		35.7
Ceftolozane/tazobactam	>16	>16	0.25 to >16	2.6[c]	1.5	95.9	2.6		97.4
Aztreonam	>16	>16	≤0.03 to >16	7.9	0.5	91.5	7.1	0.8	92.1
Ciprofloxacin	>4	>4	≤0.03 to >4	7.7	2.0	90.3	7.7	2.0	90.3
Levofloxacin	16	>32	≤0.015 to >32	10.4	6.7	82.9	10.4	6.7	82.9
Gentamicin	16	>16	≤0.12 to >16	44.1	3.7	52.1	41.9[d]		58.1
Amikacin	8	>32	0.5 to >32	64.0	9.8	26.1	53.7[d]		46.3
Minocycline	4	>32	0.5 to >32	57.8	16.0	26.2			
Tigecycline	0.5	2	≤0.06 to >8	93.4	5.5	1.1	53.1[e]		
TMP-SMX	>4	>4	≤0.12 to >4	16.5		83.5	16.5	3.1	80.4
Colistin	0.25	>8	≤0.06 to >8		73.3	26.7	73.3		26.7
K. pneumoniae (878)									
Aztreonam/avibactam[b]	0.25	0.5	≤0.03 to >16	(99.8)[b]			(99.8)[b]		
Ceftazidime/avibactam	2	>32	≤0.015 to >32	72.6		27.4	72.6		27.4
Meropenem/vaborbactam	2	>32	≤0.015 to >32	60.4	2.9	36.8	63.2		36.8
Ceftolozane/tazobactam	>16	>16	≤0.12 to >16	2.2	0.9	96.9	2.2		97.8
Aztreonam	>16	>16	≤0.03 to >16	5.1	0.1	94.8	4.9	0.2	94.9
Ciprofloxacin	>4	>4	≤0.03 to >4	5.0	0.8	94.2	5.0	0.8	94.2
Levofloxacin	32	>32	0.03 to >32	6.6	5.3	88.1	6.6	5.3	88.1
Gentamicin	>16	>16	≤0.12 to >16	43.5	3.0	53.5	41.3[d]		58.7
Amikacin	8	>32	0.5 to >32	61.8	10.6	27.6	52.5[d]		47.5
Minocycline	4	>32	0.5 to >32	56.9	17.3	25.8			
Tigecycline	0.5	2	0.12 to >8	95.0	3.9	1.1	50.7[e]		
TMP-SMX	>4	>4	≤0.12 to >4	14.6		85.4	14.6	2.5	82.9
Colistin	0.25	>8	≤0.06 to >8		73.2	26.8	73.2		26.8
E. cloacae complex (65)									
Aztreonam/avibactam[b]	0.25	1	≤0.03 to >16	(98.4)[b]			(98.4)[b]		
Ceftazidime/avibactam	>32	>32	0.25 to >32	42.2		57.8	42.2		57.8
Meropenem/vaborbactam	4	>32	0.03 to >32	57.8	10.9	31.2	68.8		31.2
Ceftolozane/tazobactam	>16	>16	0.25 to >16	4.7	0.0	95.3	4.7		95.3
Aztreonam	>16	>16	0.06 to >16	20.3	4.7	75.0	17.2	3.1	79.7
Ciprofloxacin	4	>4	≤0.03 to >4	18.8	15.6	65.6	18.8	15.6	65.6
Levofloxacin	2	32	0.03 to >32	34.4	12.5	53.1	34.4	12.5	53.1
Gentamicin	>16	>16	≤0.12 to >16	31.2	14.1	54.7	31.2[d]		68.8
Amikacin	8	>32	0.5 to >32	68.8	7.8	23.4	56.2[d]		43.8
Minocycline	4	32	0.5 to >32	70.3	6.2	23.4			
Tigecycline	0.5	2	0.12 to 4	90.6	9.4	0.0	76.6[e]		
TMP-SMX	>4	>4	≤0.12 to >4	17.2		82.8	17.2	1.6	81.2
Colistin	0.25	>8	0.12 to >8		82.5	17.5	82.5		17.5
E. coli (58)									
Aztreonam/avibactam[b]	0.25	4	≤0.03 to >16	(98.3)[b]			(98.3)[b]		
Ceftazidime/avibactam	>32	>32	≤0.015 to >32	49.2		50.8	49.2		50.8
Meropenem/vaborbactam	8	>32	≤0.015 to >32	47.5	8.5	44.1	55.9		44.1
Ceftolozane/tazobactam	>16	>16	≤0.12 to >16	6.8	5.1	88.1	6.8		93.2
Aztreonam	>16	>16	0.06 to >16	10.2	1.7	88.1	5.1	5.1	89.8
Ciprofloxacin	>4	>4	≤0.008 to >4	13.6	1.7	84.7	13.6	1.7	84.7
Levofloxacin	16	>32	≤0.015 to >32	18.6	8.5	72.9	18.6	8.5	72.9

Continued

Table 2. *Continued*

Antimicrobial agent (no. of isolates)	mg/L			CLSI/US FDA[a]			EUCAST[a]		
	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R	%S	%I	%R
Gentamicin	2	>16	0.5 to >16	57.6	0.0	42.4	54.2[d]		45.8
Amikacin	4	>32	1 to >32	86.4	1.7	11.9	76.3[d]		23.7
Minocycline	2	32	0.5 to >32	72.9	5.1	22.0			
Tigecycline	0.25	0.25	≤0.06 to 1	100.0	0.0	0.0	98.3		1.7
TMP-SMX	>4	>4	≤0.12 to >4	18.6		81.4	18.6	0.0	81.4
Colistin	0.25	0.25	0.12 to >8		98.3	1.7	98.3		1.7
S. marcescens (36)									
Aztreonam/avibactam[b]	0.25	1	0.12 to 2	(100.0)[b]			(100.0)[b]		
Ceftazidime/avibactam	1	>32	0.12 to >32	88.9		11.1	88.9		11.1
Meropenem/vaborbactam	1	16	0.03 to >32	88.9	0.0	11.1	88.9		11.1
Ceftolozane/tazobactam	>16	>16	0.5 to >16	11.1	5.6	83.3	11.1		88.9
Aztreonam	>16	>16	0.06 to >16	16.7	2.8	80.6	16.7	0.0	83.3
Ciprofloxacin	4	>4	0.03 to >4	13.9	8.3	77.8	13.9	8.3	77.8
Levofloxacin	2	32	0.06 to 32	13.9	25.0	61.1	13.9	25.0	61.1
Gentamicin	2	>16	0.25 to >16	55.6	2.8	41.7	50.0[b]		50.0
Amikacin	16	>32	1 to >32	72.2	11.1	16.7	44.4[b]		55.6
Minocycline	8	32	1 to >32	34.3	31.4	34.3			
Tigecycline	2	4	0.5 to 8	55.6	41.7	2.8	5.6[e]		
TMP-SMX	4	>4	≤0.12 to >4	38.9		61.1	38.9	22.2	38.9
Colistin	>8	>8	0.25 to >8		16.7	83.3	16.7		83.3

TMP-SMX, trimethoprim/sulfamethoxazole.
[a]Criteria as published by CLSI,[11] US FDA[17] and EUCAST.[12]
[b]Values in parentheses indicate percentage inhibited at ≤8 mg/L aztreonam/avibactam.
[c]The vast majority (89.3%) of ceftolozane/tazobactam-susceptible isolates harboured a class D β-lactamase, mainly OXA-48-like (75.0%). Only two isolates had a class A carbapenemase: one KPC-2 and one IMI-4.
[d]For infections originating from the urinary tract. For systemic infections, aminoglycosides must be used in combination with other active therapy.[12]
[e]For comparison, EUCAST breakpoints published for *E. coli* and *C. koseri* (≤0.5 mg/L) were applied for all Enterobacterales species.[12]

Table 3. Frequency of carbapenemase genes stratified by geographical region

β-Lactamase	No. of isolates (% of CREs for the region)				
	W-EU	E-EU	APAC	LATAM	All regions
KPC type	151 (66.5)	116 (25.6)	38 (21.5)	168 (70.0)	473 (43.1)
KPC-2	20 (8.8)	81 (17.8)	37 (20.9)	158 (**65.8**)	296 (27.0)
KPC-3	131 (**57.7**)	35 (7.7)	—	10 (4.2)	176 (16.0)
KPC-4	—	-	1 (0.6)	—	1 (0.1)
MBL	44 (19.4)	134[a] (29.5)	109 (61.6)	60 (25.0)	347 (31.6)
NDM type	26 (11.5)	109[a] (24.0)	101 (**57.1**)	56[b] (23.3)	292 (26.6)
VIM type	18 (7.9)	25[a] (5.5)	3 (1.7)	3 (1.3)	48 (4.4)
IMP type	—	1 (0.2)	5 (2.8)	1 (0.4)	7 (0.6)
OXA-48 type	31 (13.7)	144 (**31.7**)	29 (16.4)	1 (0.4)	205 (18.7)
≥2 β-lactamase	12 (5.3)	22 (4.8)	21 (11.9)	2 (0.8)	57 (5.2)
Total	214 (94.3)	374 (82.4)[c]	155 (87.6)	229 (95.4)	972 (88.5)
No carbapenemase	13 (5.7)	80 (17.6)	22 (12.4)	11 (4.6)	126 (11.5)

Values in bold indicates the frequency of the most common carbapenemase in the region.
[a]One isolate had an NDM-1 and a VIM-1.
[b]One isolate had an NDM-1 and an NDM-5.
[c]86.3% (69/80) of carbapenemase-negative CRE were from Poland.

(29.4%; 5/17), South Korea (20.0%; 2/10), Romania (15.3%; 2/13), Spain (10.2%; 4/39) and Turkey (9.6%; 14/146).

A carbapenemase gene was not identified in 126 (11.5%) CRE isolates. Carbapenemase-negative CRE isolates were mostly *K. pneumoniae* (66.7%; 84/126), followed by *E. coli* (11.9%), *Klebsiella aerogenes* (9.5%), *E. cloacae* complex (8.7%) and *S. marcescens* (3.2%). Interestingly, the percentage of carbapenem-resistant isolates that were carbapenemase negative were markedly higher among *K. aerogenes* (80.0%) compared with the other species listed above (9.6% to 25.9%). Moreover, most carbapenemase-negative CRE isolates were from E-EU (80 of 126), mainly from Poland ($n=69$); 74.2% (69/93) of CRE isolates from Poland were carbapenemase negative.

Aztreonam/avibactam activity was very consistent across the geographical regions evaluated (Table 4). Aztreonam/avibactam $MIC_{50/90}$ values were 0.25/0.5 mg/L in W-EU, E-EU and LATAM, and 0.12/0.5 mg/L in APAC. All isolates from W-EU and LATAM were inhibited at an aztreonam/avibactam MIC of ≤8 mg/L (Table 4). Notably, aztreonam/avibactam inhibited 100.0% of MBL producers ($MIC_{50/90}$, 0.12/0.5 mg/L) and OXA-48-like producers ($MIC_{50/90}$, 0.25/0.5 mg/L) at ≤4 and ≤8 mg/L, respectively (Table 1 and 4). Of the 57 isolates that carried more than one carbapenemase gene, 97.6% were inhibited at an aztreonam/avibactam MIC of ≤8 mg/L ($MIC_{50/90}$, 0.25/0.5 mg/L; Table 1). CRE isolates with aztreonam/avibactam MICs >8 mg/L were observed only in Poland [an *E. coli* and an *Enterobacter hormaechei* (*E. cloacae* complex), both collected in 2020), Taiwan (a *K. pneumoniae* from 2021) and Thailand (a *K. pneumoniae* from 2019; Table S2).

Ceftazidime/avibactam exhibited good activity (70.3%–80.2% susceptibility) against CRE isolates from W-EU, E-EU and LATAM, but limited activity against CRE isolates from APAC (39.5% susceptibility; Table 4). Moreover, ceftazidime/avibactam exhibited good activity against KPC producers from all regions (89.5%–99.4% susceptible) as well as OXA-48-like producers from W-EU (87.1% susceptible), E-EU (91.0% susceptible) and LATAM (100% susceptible; Table 4). The limited activity of ceftazidime/avibactam against OXA-48-like producers from APAC (37.9% susceptible) was mainly because 62.1% (18/29) of these isolates co-produced an NDM (data not shown).

Meropenem/vaborbactam exhibited good activity against CRE isolates from W-EU (77.5%/81.1% susceptible per CLSI/EUCAST) and LATAM (78.8%/83.3% susceptible per CLSI/EUCAST), but limited activity against CRE isolates from both E-EU (50.1%/53.0% susceptible per CLSI/EUCAST) and APAC (40.3%/46.0% susceptible per CLSI/EUCAST; Table 4). The limited activity of meropenem/vaborbactam in E-EU and APAC can be justified by the high prevalence of OXA-48-like in E-EU and MBLs in APAC (Table 3). It is also important to note that 15.3%/23.4% of MBL producers were categorized as susceptible to meropenem/vaborbactam per CLSI/EUCAST criteria, and susceptibility rates were higher in W-EU (27.3%/29.5%) and LATAM (21.7/38.3%; Table 4). Since vaborbactam does not effectively inhibit MBLs, meropenem/vaborbactam susceptibility among MBL producers can be explained mainly by low expression of the MBL gene, low hydrolysis of meropenem by the MBL, or a combination of these two factors. Another important point to be considered is the higher breakpoint for meropenem/vaborbactam compared with meropenem alone. Among the MBL-producing, meropenem/vaborbactam-susceptible isolates evaluated in this study, 88.8% and 83.8% had MIC values ≤8 mg/L for meropenem and imipenem, respectively (data not shown).

Tigecycline susceptibility rates ranged from 92.1% to 94.7% when US FDA breakpoints were applied but varied from 47.6% to 59.3% when EUCAST breakpoints for *E. coli* and *C. koseri* were applied.[12] Colistin susceptibility rates per EUCAST criteria varied widely from 90.3% in W-EU to only 60.5% in LATAM. Moreover, the *in vitro* activity of comparator agents also varied broadly according to the carbapenemase produced and the antimicrobial resistance phenotype (Table 4).

A summary of the MIC results from the characterization of the four isolates exhibiting aztreonam/avibactam MIC values >8 mg/L is shown in Table S2. A four amino acid insertion (YRIK) in the PBP3 and a CMY-141-encoding gene were detected in the *E. coli* strain 1183154 from Poland. The *E. cloacae* species complex isolate 1183311, also from Poland, was identified as *E. hormaechei*; it overexpressed *act-17* and carried $bla_{CTX\text{-}M\text{-}15}$. This isolate had a premature stop codon within OmpF and alterations in OmpC and PBP3.

The *K. pneumoniae* isolate 1116221 from Thailand carried $bla_{DHA\text{-}1}$ and had a premature stop codon at position 43 of OmpK36, whereas a WT sequence was observed for PBP3. Lastly, the *K. pneumoniae* isolate 1215485 from Taiwan carried KPC-2 and a VEB variant with a single amino acid substitution at residue 75 when compared with VEB-1 (VEB-1_{P75L}), designated $bla_{VEB\text{-}31}$. This isolate also had multiple alterations in the OmpK36, whereas WT sequences were observed for PBP3. Finally, the main β-lactamase genes (i.e., bla_{CMY141}, $bla_{CTX\text{-}M\text{-}15}$, $bla_{DHA\text{-}1}$ and $bla_{KPC\text{-}2}$) detected in each of these four isolates with elevated aztreonam/avibactam MICs were present in single copies (Table S2).

Discussion

The spread of CRE represents a major challenge for antimicrobial treatment worldwide. While the approval of new BL/BLI compounds in the last decade, such as ceftazidime/avibactam, meropenem/vaborbactam and imipenem/relebactam, represented remarkable progress for the treatment of infections caused by CRE, their spectrum of activity is reduced in regions where MBL-producing Enterobacterales are prevalent.[18] Furthermore, because these newer BL/BLIs lack activity against MBL-producing organisms, the increased use of these compounds, especially in regions where serine carbapenemases represent the main mechanisms of carbapenem resistance among CREs, may increase the occurrence of MBL-producing and non-carbapenemase-producing CREs.[19]

Cefiderocol is the only β-lactam compound with activity against MBL-producing strains, including those that co-produce ESBLs and/or serine carbapenemases, currently approved by the US FDA and EMA.[20] The absence of cefiderocol in this investigation is a limitation of the study. Other compounds with *in vitro* activity against MBL-producing CREs include colistin, tigecycline, fosfomycin, minocycline and some aminoglycosides; however, these compounds have significant toxicity issues and/or spectrum deficiencies that prevent their use as empirical or guided treatment for life-threatening infections.[2]

Table 4. Activity of aztreonam/avibactam and comparator antimicrobial agents tested against 1098 CRE isolates collected worldwide (ex-USA) in 2019–21 stratified by region and β-lactamase type

	% Susceptible per CLSI/EUCAST[a]									
Antimicrobial agent	W-EU		E-EU		APAC		LATAM		All regions	
CRE, *n*	227		454		177		240		1098	
Aztreonam/avibactam[b]	(100.0)[b]		(99.6)[b]		(98.9)[b]		(100.0)[b]		(99.6)[b]	
Ceftazidime/avibactam	80.2/	80.2	70.3/	70.3	39.5/	39.5	74.1/	74.1	68.2/	68.2
Meropenem/vaborbactam	77.5/	81.1	50.1/	53.0	40.3/	46.0	78.8/	83.3	60.5/	64.3
Aztreonam	8.8/	7.9	8.8/	8.1	5.6/	5.1	7.1/	5.8	7.9	7.1
Amikacin	70.5/	65.6[c]	50.4/	37.4[c]	89.8/	80.8[c]	64.6/	53.3[c]	64.0/	53.7[c]
Gentamicin	54.9/	54.0[c]	40.7/	38.1[c]	48.6/	47.5[c]	37.1/	33.8 [c]	44.1/	41.9[c]
Levofloxacin	13.3/	13.3	7.3/	7.3	9.6/	9.6	14.2/	14.2	10.4/	10.4
Minocycline	64.8/	NA[d]	52.2/	NA[d]	55.1/	NA[d]	64.9/	NA[d]	58.1/	NA[d]
Tigecycline[e]	94.7/	55.9	93.6/	47.6	93.2/	59.3	92.1/	56.2	93.4/	53.1
Colistin	NA[d]/	90.3	NA[d]/	69.1	NA[d]/	79.7	NA[d]/	60.5	NA[d]/	73.3
KPC producers, *n*	151		116		38		168		473	
Aztreonam/avibactam[b]	(100.0)[b]		(100.0)[b]		(97.4)[b]		(100.0)[b]		(99.8)[b]	
Ceftazidime/avibactam	94.7/	94.7	94.8/	94.8	89.5/	89.5	99.4/	99.4	96.0/	96.0
Meropenem/vaborbactam	92.1/	94.7	95.7/	96.6	92.1/	92.1	98.8/	99.4	95.3/	96.6
Aztreonam	0.0/	0.0	0.0/	0.0	0.0/	0.0	0.0/	0.0	0.0/	0.0
Amikacin	67.5/	64.2[c]	42.2/	33.6[c]	92.1/	89.5[c]	70.2/	59.5[c]	64.3/	57.1[c]
Gentamicin	54.0/	52.7[c]	58.6/	54.3[c]	39.5/	39.5[c]	38.7/	36.3[c]	48.5/	46.2[c]
Levofloxacin	10.7/	10.7	1.7/	1.7	5.3/	5.3	9.5/	9.5	7.6/	7.6
Minocycline	68.9/	NA[d]	59.5/	NA[d]	63.2/	NA[d]	71.9/	NA[d]	67.2/	NA[d]
Tigecycline[e]	96.7/	55.6	94.0/	49.1	100.0/	42.1	91.7/	58.9	94.5/	54.1
Colistin	NA[d]/	91.3	NA[d]/	70.7	NA[d]/	55.3	NA[d]/	53.6	NA[d]/	70.0
MBL producers, *n*	44		134		109		60		347	
Aztreonam/avibactam[b]	(100.0)[b]		(100.0)[b]		(100.0)[b]		(100.0)[b]		(100.0)[b]	
Ceftazidime/avibactam	0.0/	0.0	0.7/	0.7	5.5/	5.5	0.0/	0.0	2.0/	2.0
Meropenem/vaborbactam	27.3/	29.5	11.2/	16.4	12.0/	21.3	21.7/	38.3	15.3/	23.4
Aztreonam	20.5/	18.2	14.9/	14.2	8.3/	7.3	26.7/	23.3	15.6/	14.1
Amikacin	65.9/	59.1 [c]	50.0/	23.9[c]	87.2/	77.1[c]	48.3/	33.3 [c]	63.4/	46.7[c]
Gentamicin	40.9/	40.9 [c]	31.3/	26.9[c]	48.6/	46.8[c]	30.0/	23.3[c]	37.8/	34.3[c]
Levofloxacin	11.4/	11.4	9.0/	9.0	8.3/	8.3	26.7/	26.7	12.1/	12.1
Minocycline	63.6/	NA[d]	65.7/	NA[d]	59.3/	NA[d]	50.0/	NA[d]	60.7/	NA[d]
Tigecycline[e]	90.9/	56.8	93.3/	51.5	93.6/	70.6	95.0/	55.0	93.4/	58.8
Colistin	NA[d]/	90.9	NA[d]/	76.7	NA[d]/	86.2	NA[d]/	76.7	NA[d]/	81.5
OXA-48-like producers, *n*	31		144		29		1		205	
Aztreonam/avibactam[b]	(100.0)[b]		(100.0)[b]		(100.0)[b]		(100.0)[b]		(100.0)[b]	
Ceftazidime/avibactam	87.1/	87.1	91.0/	91.0	37.9/	37.9	100.0/	100.0	82.9/	82.9
Meropenem/vaborbactam	54.8/	61.3	22.2/	22.9	6.9/	6.9	0.0/	0.0	24.9/	26.3
Aztreonam	29.0/	25.8	13.9/	13.9	10.3/	10.3	0.0/	0.0	15.6/	15.6
Amikacin	83.9/	80.6[c]	36.8/	30.6[c]	82.8/	62.1[c]	100.0/	100.0[c]	50.7/	42.9[c]
Gentamicin	61.3/	61.3[c]	29.2/	27.8[c]	72.4/	72.4[c]	0.0/	0.0[c]	40.0/	39.0[c]
Levofloxacin	16.1/	16.1	9.0/	9.0	0.0/	0.0	0.0/	0.0	8.8/	8.8
Minocycline	61.3/	NA[d]	34.7/	NA[d]	20.7/	NA[d]	0.0/	NA[d]	36.6/	NA[d]
Tigecycline[e]	87.1/	61.3	96.5/	35.4	96.6/	55.2	0.0/	0.0	94.6/	42.0
Colistin	NA[d]/	83.9	NA[d]/	60.4	NA[d]/	72.4	NA[d]/	0.0	NA[d]/	65.4
No carbapenemase, *n*	13		80		22		11		126	
Aztreonam/avibactam[b]	(100.0)[b]		(97.5)[b]		(95.5)[b]		(100.0)[b]		(97.6)[b]	
Ceftazidime/avibactam	92.3/	92.3	97.5/	97.5	86.4/	86.4	81.8/	81.8	93.7/	93.7
Meropenem/vaborbactam	92.3/	100.0	91.1/	96.2	95.5/	95.5	90.9/	90.9	92.0/	96.0
Aztreonam	15.4/	15.4	2.5/	0.0	0.0/	0.0	0.0/	0.0	3.2	1.6
Amikacin	86.4/	69.2[c]	78.8/	70.0[c]	90.9/	81.8[c]	63.6/	63.6[c]	80.2/	71.4[c]

Continued

Table 4. *Continued*

Antimicrobial agent	% Susceptible per CLSI/EUCAST[a]									
	W-EU		E-EU		APAC		LATAM		All regions	
Gentamicin	69.2/	69.2[c]	46.2/	46.2[c]	54.5/	54.5[c]	45.5/	45.5[c]	50.0/	50.0[c]
Levofloxacin	38.5/	38.5	7.5/	7.5	27.3/	27.3	18.2/	18.2	15.1/	15.1
Minocycline	38.5/	NA[d]	48.8/	NA[d]	40.9/	NA[d]	45.5/	NA[d]	46.0/	NA[d]
Tigecycline[e]	100.0/	53.8	88.8/	58.8	77.3/	50.0	90.9/	27.3	88.1/	54.0
Colistin	NA[d]/	92.3	NA[d]/	72.5	NA[d]/	90.9	NA[d]/	81.8	NA[d]/	78.6
CZA-R (>8 mg/L), *n*	45		135		107		62		349	
Aztreonam/avibactam[b]	(100.0)[b]		(98.5)[b]		(98.1)[b]		(100.0)[b]		(98.9)[b]	
Meropenem/vaborbactam	27.3/	29.5	11.1/	16.3	13.1/	20.6	23.0/	39.3	15.9/	23.3
Aztreonam	20.0/	17.8	14.8/	14.1	6.5/	5.6	25.8/	22.6	14.9/	13.5
Amikacin	64.4/	57.8[c]	51.1/	25.2[c]	87.9/	77.6[c]	48.4/	33.9[c]	63.6/	47.0[c]
Gentamicin	40.0/	40.0[c]	31.9/	27.4[c]	52.3/	50.5[c]	30.6/	24.2[c]	39.0/	35.5[c]
Levofloxacin	11.1/	11.1	9.0/	9.0	7.5/	7.5	27.4/	27.4	12.1/	12.1
Minocycline	62.2/	NA[d]	65.9/	NA[d]	57.9/	NA[d]	50.0/	NA[d]	60.2/	NA[d]
Tigecycline[e]	91.1/	55.6	93.3/	51.9	91.6/	70.1	95.2/	56.5	92.8/	58.7
Colistin	NA[d]/	91.1	NA[d]/	76.9	NA[d]/	86.9	NA[d]/	77.4	NA[d]/	81.9
Colistin-R (>2 mg/L), *n*	22		140		36		94		292	
Aztreonam/avibactam[b]	(100.0)[b]		(100.0)[b]		(97.2)[b]		(100.0)[b]		(99.7)[b]	
Ceftazidime/avibactam	81.8/	81.8	77.9/	77.9	61.1/	61.1	85.1/	85.1	78.4/	78.4
Meropenem/vaborbactam	68.2/	72.7	43.6/	46.4	68.6/	68.6	84.9/	89.2	61.7/	64.8
Aztreonam	18.2/	18.2	8.6/	8.6	2.8/	2.8	7.4/	7.4	8.2/	8.2
Amikacin	36.4/	36.4[c]	42.1/	31.4[c]	83.3	72.2[c]	43.6/	30.9[c]	47.3/	36.6[c]
Gentamicin	63.6/	59.1[c]	30.0/	30.0[c]	38.9/	38.9[c]	30.9/	26.6[c]	33.9/	32.2[c]
Levofloxacin	4.5/	4.5	5.7/	5.7	5.6/	5.6	8.5/	8.5	6.5/	6.5
Minocycline	27.3/	NA[d]	40.7/	NA[d]	40.0/	NA[d]	62.4/	NA[d]	46.6/	NA[d]
Tigecycline[e]	86.4/	13.6	92.1/	32.1	91.7/	33.3	83.0/	41.5	88.7/	33.9
Tigecycline-NS (>0.5 mg/L)[e], *n*	100		238		72		105		515	
Aztreonam/avibactam[b]	(100.0)[b]		(99.6)[b]		(98.6)[b]		(100.0)[b]		(99.6)[b]	
Ceftazidime/avibactam	80.0/	80.0	72.7/	72.7	55.6/	55.6	74.3/	74.3	72.0/	72.0
Meropenem/vaborbactam	75.8/	79.8	42.4/	46.2	50.7/	52.2	72.1/	77.9	56.1/	60.0
Aztreonam	7.0/	6.0	10.5/	10.5	5.6/	4.2	5.7/	5.7	8.2/	7.8
Amikacin	63.0/	59.0[c]	40.8/	29.0[c]	87.5/	80.6[c]	61.9/	48.6[c]	55.9/	46.0[c]
Gentamicin	57.0/	56.0[c]	30.7/	28.6[c]	45.8/	45.8[c]	36.2/	31.4[c]	39.0/	36.9[c]
Levofloxacin	6.0/	6.0	3.8/	3.8	6.9/	6.9	1.9/	1.9	4.3/	4.3
Minocycline	30.0/	NA[d]	27.3/	NA[d]	23.2/	NA[d]	39.4/	NA[d]	29.7/	NA[d]
Colistin	NA[d]/	80.8	NA[d]/	59.9	NA[d]/	66.7	NA[d]/	47.6	NA[d]/	62.4

CZA, ceftazidime/avibactam; R, resistant; NS, non-susceptible.
[a]Criteria as published by CLSI,[11] EUCAST[12] and US FDA.[17]
[b]Values in brackets indicate percentage inhibited at ≤8 mg/L aztreonam/avibactam.
[c]For infections originating from the urinary tract. For systemic infections, aminoglycosides must be used in combination with other active therapy.[12]
[d]NA, not applicable; breakpoint has not been published.[11,12]
[e]US FDA breakpoints were applied due to the absence of CLSI breakpoints for this compound.[11] Moreover, EUCAST breakpoints published for *E. coli* and *C. koseri* (≤0.5 mg/L) were applied for all Enterobacterales species for comparison.[12]

Decreased susceptibility to aztreonam/avibactam (MIC, >8 mg/L) was observed in only four CRE isolates, two *K. pneumoniae*, one *E. coli* and one *E. hormaechei* (*E. cloacae* complex), which showed an MDR profile (Table S2). The BL and BL/BLI MIC results obtained against *K. pneumoniae* 1116221 were likely due to the production of DHA-1 combined with the lack of OmpK36, as previously described.[21] Decreased susceptibility to aztreonam/avibactam in *E. coli* isolates was reported by other investigators and seemed to be caused mostly by the association of PBP3 alterations with the production of CMY β-lactamases, similar to what was observed here with *E. coli* 1183154.[22,23] Furthermore, *E. coli* 1183154 produced altered OmpC and OmpF, which compromised the entry of BLs, including carbapenems.[24] The decreased susceptibility of *E. hormaechei* 1183311

to aztreonam/avibactam could be due to the absence of OmpF and hyperproduction of AmpC (ACT-17). Although the mutations observed in the OmpC and PBP3 could also provide a deleterious effect on the activity of this combination, this remains to be elucidated.

K. pneumoniae 1215485 lacked OmpK35 and showed numerous mutations within OmpK36, but their effect on aztreonam/avibactam MIC is not well understood. However, the lack of OmpK35 and alterations in OmpK36 could compromise the access of BL agents other than carbapenems to the periplasmic space and act synergistically with the presence of both bla_{KPC-2} and the novel bla_{VEB-31} on the decreased susceptibility to various BL agents. However, these resistance mechanisms could not completely explain the elevated MIC for aztreonam/avibactam and ceftazidime/avibactam, as avibactam should inhibit both KPC-2 and VEB-31. The VEB-31 presented here was fully conserved at 8 of 12 key residues that form the avibactam-binding pocket in class A β-lactamases based on the CTX-M-15 crystal structure.[25] Compared with VEB-1, VEB-31 had an altered amino acid that resided close to the serine active site (S^{70}XXK). Studies are underway to help elucidate whether this alteration could jeopardize avibactam binding. Nevertheless, a synergistic effect of multiple resistance mechanisms seems to be necessary to increase the aztreonam/avibactam MIC, but these mechanisms vary markedly by bacterial species, as shown here and elsewhere.[21,22,26]

In the present study, we evaluated the *in vitro* activity of aztreonam/avibactam against a large collection of CRE isolates ($n=1098$) obtained from a contemporary (2019–21) collection of 24 924 consecutively collected Enterobacterales isolates from 69 medical centres located in 36 countries. Aztreonam/avibactam was active against 99.6% of the CRE isolates, including 100.0% of the MBL producers and 98.9% of the ceftazidime/avibactam-resistant isolates. Our results corroborate previous publications by other investigators.[27–29] Moreover, although we did not include US CRE isolates in our study, the activity of aztreonam/avibactam was recently evaluated against MBL-producing CRE isolates from the USA by investigators from the CDC.[30] Bhatnagar *et al.*[30] evaluated 64 MBL-producing, ceftazidime/avibactam-resistant isolates collected from 24 states by four Antimicrobial Resistance Laboratory Network regional laboratories. Aztreonam/avibactam $MIC_{50/90}$ values were 0.5/8 mg/L and 96.9% (62/64) of isolates were inhibited at ≤8 mg/L aztreonam/avibactam. Thus, these results, coupled with results published by other investigators, indicate that aztreonam/avibactam provides worldwide coverage of CRE.

In summary, aztreonam/avibactam demonstrated potent activity against a large collection of contemporary CRE isolates, including MBL producers and ceftazidime/avibactam-resistant isolates. The results of this large international investigation support the clinical development of aztreonam/avibactam.

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Transparency declarations

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Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC-AMR* Online.

References

1 Bush K, Bradford PA. Epidemiology of β-lactamase-producing pathogens. *Clin Microbiol Rev* 2020; **33**: e00047. https://doi.org/10.1128/CMR.00047-19

2 Tompkins K, van Duin D. Treatment for carbapenem-resistant *Enterobacterales* infections: recent advances and future directions. *Eur J Clin Microbiol Infect Dis* 2021; **40**: 2053–68. https://doi.org/10.1007/s10096-021-04296-1

3 Cruz-Lopez F, Marinez-Melendez A, Morfin-Otero R *et al*. Efficacy and *in vitro* activity of novel antibiotics for infections with carbapenem-resistant Gram-negative pathogens. *Front Cell Infect Microbiol* 2022; **12**: 884365. https://doi.org/10.3389/fcimb.2022.884365

4 Falcone M, Daikos G, Tiseo G *et al*. Efficacy of ceftazidime-avibactam plus aztreonam in patients with bloodstream infections caused by metallo-β-lactamase–producing Enterobacterales. *Clin Infect Dis* 2021; **72**: 1871–8. https://doi.org/10.1093/cid/ciaa586

5 Mauri C, Maraolo A, Di Bella S *et al*. The revival of aztreonam in combination with avibactam against metallo-β-lactamase-producing Gram-negatives: a systematic review of *in vitro* studies and clinical cases. *Antibiotics (Basel)* 2021; **10**: 1012. https://doi.org/10.3390/antibiotics10081012

6 Brogden RN, Aztreonam HR. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* 1986; **31**: 96–130. https://doi.org/10.2165/00003495-198631020-00002

7 Soriano A, Carmeli Y, Omrani A *et al*. Ceftazidime-avibactam for the treatment of serious Gram-negative infections with limited treatment options: a systematic literature review. *Infect Dis Ther* 2021; **10**: 1989–2034. https://doi.org/10.1007/s40121-021-00507-6

8 Fuhrmeister AS, Jones RN, Sader HS *et al*. Global surveillance of antimicrobial resistance: 20 years of experience with the SENTRY program. *Open Forum Infect Dis* 2019; **6**: S1–S102. https://doi.org/10.1093/ofid/ofy346

9 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Eleventh Edition: M07*. 2018.

10 Cornely OA, Cisneros JM, Torre-Cisneros J *et al*. Pharmacokinetics and safety of aztreonam/avibactam for the treatment of complicated intra-abdominal infections in hospitalized adults: results from the REJUVENATE study. *J Antimicrob Chemother* 2020; **75**: 618–27. https://doi.org/10.1093/jac/dkz497

11 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing—Thirty-second Edition: M100Ed33*. 2022.

12 EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0. 2022. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_12.0_Breakpoint_Tables.pdf

13 Bankevich A, Nurk S, Antipov D *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Compt Biol* 2012; **19**: 455–77. https://doi.org/10.1089/cmb.2012.0021

14 Camacho C, Coulouris G, Avagyan V *et al*. Blast+: architecture and applications. *BMC Bioinformatics* 2009; **10**: 421. https://doi.org/10.1186/1471-2105-10-421

15 Castanheira M, Costello SE, Woosley LN *et al*. Evaluation of clonality and carbapenem resistance mechanisms among *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex and *Enterobacteriaceae* isolates collected in European and Mediterranean countries and detection of two novel β-lactamases, GES-22 and VIM-35. *Antimicrob Agents Chemother* 2014; **58**: 7358–66.

16 Castanheira M, Doyle TB, Hubler C *et al*. Ceftazidime-avibactam activity against a challenge set of carbapenem-resistant *Enterobacterales*: Ompk36 L3 alterations and β-lactamases with ceftazidime hydrolytic activity lead to elevated MIC values. *Int J Antimicrob Agents* 2020; **56**: 106011. https://doi.org/10.1016/j.ijantimicag.2020.106011

17 FDA. Antibacterial susceptibility test interpretive criteria. https://www.fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria

18 Jean S, Harnod D, Hsueh P. Global threat of carbapenem-resistant Gram-negative bacteria. *Front Cell Infect Microbiol* 2022; **12**: 823684. https://doi.org/10.3389/fcimb.2022.823684

19 Bouganim R, Dykman L, Fakeh O *et al*. The clinical and molecular epidemiology of noncarbapenemase-producing carbapenem-resistant Enterobacteriaceae: a case-case-control matched analysis. *Open Forum Infect Dis* 2020; **7**: ofaa299. https://doi.org/10.1093/ofid/ofaa299

20 Timsit J, Paul M, Shields R *et al*. Cefiderocol for the treatment of infections due to metallo-B-lactamase-producing pathogens in the CREDIBLE-CR and APEKS-NP phase 3 randomized studies. *Clin Infect Dis* 2022. https://doi.org/10.1093/cid/ciac078

21 Mendes R, Doyle T, Streit J *et al*. Investigation of mechanisms responsible for decreased susceptibility of aztreonam/avibactam activity in clinical isolates of Enterobacterales collected in Europe, Asia and Latin America in 2019. *J Antimicrob Chemother* 2021; **76**: 2833–8. https://doi.org/10.1093/jac/dkab279

22 Sadek M, Juhas M, Poirel L *et al*. Genetic features leading to reduced susceptibility to aztreonam-avibactam among metallo-β-lactamase-producing *Escherichia coli* isolates. *Antimicrob Agents Chemother* 2020; **64**: e01659. https://doi.org/10.1128/AAC.01659-20

23 Sader HS, Mendes RE, Arends SJR *et al*. Antimicrobial activities of aztreonam-avibactam and comparator agents tested against Enterobacterales from European hospitals analysed by geographic region and infection type (2019-2020). *Eur J Clin Microbiol Infect Dis* 2022; **41**: 477–87. https://doi.org/10.1007/s10096-022-04400-z

24 Domenech-Sanchez A, Martinez-Martinez L, Hernandez-Alles S *et al*. Role of *Klebsiella pneumoniae* OmpK35 porin in antimicrobial resistance. *Antimicrob Agents Chemother* 2003; **47**: 3332–5. https://doi.org/10.1128/AAC.47.10.3332-3335.2003

25 Lahiri SD, Alm RA. Identification of novel VEB β-lactamase enzymes and their impact on avibactam inhibition. *Antimicrob Agents Chemother* 2016; **60**: 3183–6. https://doi.org/10.1128/AAC.00047-16

26 Estabrook M, Kazmierczak K, Wise M *et al*. Molecular characterization of clinical isolates of Enterobacterales with elevated MIC values for aztreonam-avibactam from the INFORM global surveillance study, 2012-2017. *J Glob Antimicrob Resist* 2021; **24**: 316–20. https://doi.org/10.1016/j.jgar.2021.01.010

27 Karlowsky JA, Kazmierczak KM, de Jonge BLM *et al*. *In vitro* activity of aztreonam-avibactam against *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolated by clinical laboratories in 40 countries from 2012 to 2015. *Antimicrob Agents Chemother* 2017; **61**: e00472-17. https://doi.org/10.1128/AAC.00472-17

28 Wei J, Zou C, Wang D *et al*. Genetic diversity and in vitro activity of ceftazidime/avibactam and aztreonam/avibactam against imipenem-resistant Enterobacteriaceae isolates in Southwest China: a single-centre study. *J Glob Antimicrob Resist* 2020; **22**: 448–51. https://doi.org/10.1016/j.jgar.2020.04.023

29 Mischnik A, Baumert P, Hamprecht A *et al*. Susceptibility to cephalosporin combinations and aztreonam/avibactam among third-generation cephalosporin-resistant Enterobacteriaceae recovered on hospital admission. *Int J Antimicrob Agents* 2017; **49**: 239–42. https://doi.org/10.1016/j.ijantimicag.2016.10.013

30 Bhatnagar A, Boyd S, Sabour S *et al*. Aztreonam-avibactam susceptibility testing program for metallo-beta-lactamase-producing *Enterobacterales* in the antibiotic resistance laboratory network, March 2019 to December 2020. *Antimicrob Agents Chemother* 2021; **65**: e00486-21. https://doi.org/10.1128/AAC.00486-21