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In vitro activity of aztreonam-avibactam and comparators against Metallo-β-Lactamase-producing Enterobacterales from ATLAS Global Surveillance Program, 2016–2020

Gian Maria Rossolini[a,*], Francis F. Arhin[b], Michal Kantecki[c]

[a] Department of Experimental and Clinical Medicine, University of Florence, Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy
[b] Pfizer Inc., Kirkland, Canada
[c] Pfizer Inc., Paris, France

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ABSTRACT

Objectives: Metallo-β-lactamase (MBL)-producing Enterobacterales are a major challenge worldwide due to limited treatment options. Aztreonam-avibactam (ATM-AVI), which is under clinical development, has shown activity against MBL-positive isolates. This study evaluated the prevalence of MBL producers and the nature of enzymes among a global collection of clinical isolates of Enterobacterales from the Antimicrobial Testing Leadership and Surveillance program (ATLAS) surveillance program (2016–2020), and the antimicrobial activity of ATM-AVI and comparators against this collection.

Methods: Non-duplicate clinical isolates of Enterobacterales (N = 106 686) collected across 63 countries were analysed. Antimicrobial susceptibility was performed using broth microdilution. Minimum inhibitory concentrations (MICs) were interpreted using Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing breakpoints. Provisional pharmacokinetic/pharmacodynamic breakpoint of ≤8 mg/L was considered for ATM-AVI. β-lactamase genes were characterized by polymerase chain reaction and sequencing. The Cochran Armitage Trend test was used to determine significant trends in percentage of isolates over time.

Results: Overall, MBL-positive isolates were 1.6% of total Enterobacterales isolates globally, with a significant increasing trend observed over time, globally and across regions ($P < 0.05$). New Delhi MBL (NDM) was the most common MBL (83.3%). ATM-AVI demonstrated potent activity against MBL-positive isolates (MIC ≤8 mg/L: 99.4% isolates inhibited; MIC_{90}, 1 mg/L). Consistent activity was also noted across different regions. Potent activity was demonstrated against different NDM variants and MBL-positive isolates co-carrying other carbapenemases (98.1% and 99.7% isolates inhibited at ≤8 mg/L, respectively). About 0.6% MBL-positive isolates (10/1707) had MICs >8 mg/L for ATM-AVI.

Conclusion: ATM-AVI demonstrated potent activity against MBL-positive isolates, including NDM variants and MBL-positive isolates co-carrying other carbapenemases, and may represent a good option for treating infections caused by MBL-positive Enterobacterales.

1. Introduction

Carbapenems remain one of the preferred choices of treatment for severe infections caused by Enterobacterales resistant to first-line agents (e.g. expanded-spectrum cephalosporins and older β-lactam and β-lactamase inhibitor combinations) [1,2]. However, over recent years, carbapenem resistance has emerged among Enterobacterales, mostly due to the acquisition of carbapenemases encoded by transferable plasmids, and currently represents a major public health problem [1–3]. A recent systematic review and meta-analysis reported prevalence of carbapenem-resistant *Klebsiella pneumoniae* (CRKp) colonization ranging from 0.13%–22% among patients tested at hospital admission, and incidence of CRKp colonization ranging from 2%–73% among hospitalized patients who were negative on admission in different settings

* Corresponding author. Mailing address: Department of Experimental and Clinical Medicine, University of Florence, c/o SOD Microbiologia e Virologia, Azienda Ospedaliera Universitaria Careggi, Largo Brambilla, 50134 Firenze, Italy.
E-mail address: gianmaria.rossolini@unifi.it (G.M. Rossolini).

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[4]. Another systematic review and meta-analysis of infections caused by carbapenem-resistant Enterobacterales (CRE) reported death rates of 26%–44%, which were overall higher than those of infections caused by carbapenem-susceptible strains (relative risk 2.05, 95% CI 1.56–2.69; $P < 0.1$) [5].

A diversity of carbapenemases has emerged in Enterobacterales including both serine-enzymes of Ambler's class A (e.g. KPC, IMI) and class D (e.g. OXA-48-like) and metallo-enzymes of class B (e.g. IMP, VIM, NDM) [6]. Among these, metallo-β-lactamases (MBLs) represent molecularly the most diverse carbapenemases [7]. New antibiotics active against CRE exhibit different activity profile depending on the resistance mechanism [8]. Hence, the discernment of the nature of CRE and their resistance mechanisms is crucial to determine the most appropriate therapeutic option [6].

There exists geographic variability in the prevalence of MBLs worldwide, with a higher prevalence in Southeast Asia compared to other geographic areas [6]. A systematic review and meta-analysis in South Asia conducted between 2010 and 2019, showed a high prevalence of MBLs among *Escherichia* coli isolates ($N = 6066$; pooled prevalence, 17% [95% CI, 12%–24%]) and reported NDM as the most prevalent carbapenemases (35%, 95% CI: 23%–49%) followed by VIM (7%, 95% CI: 3%–13%) and IMP (5%, 95% CI 2%–16%). Among the NDM-positive isolates, the NDM-1 variant was the most prevalent (33%, 95% CI: 20%–50%) [9].

MBLs have a unique zinc ion-based catalytic mechanism. MBLs (NDM, VIM, and IMP) exhibit a broad substrate specificity including penicillins, carbapenems, and cephalosporins. Moreover, MBLs are not inhibited by currently available β-lactamase inhibitors namely clavulanate, sulbactam, tazobactam, avibactam, relebactam, and vaborbactam [2]. Furthermore, MBL genes are often linked to other antibiotic resistant genes on plasmids or other mobile genetic elements and may be associated with bacterial clones which have already accumulated additional resistance mechanisms [2,10]. Consequently, treatment of infections caused by MBL-positive strains is challenging, with few remaining options that retain variable activity including aminoglycosides (e.g. gentamicin, amikacin, plazomicin), polymyxins (colistin), fosfomycin, tetracyclines (eravacycline, tigecycline), and cefiderocol [2]. However, some of these agents, especially aminoglycosides and polymyxins, are associated with safety concerns and poor diffusion in some body sites [11,12], while clinical experience with cefiderocol is still limited [2].

Aztreonam (a monobactam) is stable for hydrolysis by MBLs. However, since MBL-positive strains commonly coproduce other β-lactamases, aztreonam alone is often inactive against these strains. Avibactam, a non-β-lactam β-lactamase inhibitor, has the potential to inhibit Ambler class A, class C, and some class D β-lactamases [2]. Hence, the novel antimicrobial combination of aztreonam-avibactam (ATM-AVI) may be a potential treatment option against MBL-positive strains [13,14].

Previous global studies have reported potent antimicrobial activity of ATM-AVI against clinical isolates of Enterobacterales carrying MBL genes [13,15], including MBL-positive isolates co-carrying other carbapenemases (KPC and OXA-48) [13,16]. Recent studies confirmed consistent activity for ATM-AVI across various geographic regions and infection types [14,16,17]. However, these studies covered periods of 1–2 years [13] or specific geographical regions [14,16,17]. Moreover, there are limited studies reporting global data on ATM-AVI activity against MBL-positive isolates with a focus on NDM-variants and isolates co-producing other carbapenemases [13].

In this work, we conducted a detailed analysis over a 5-year period (2016–2020) using data from the Antimicrobial Testing Leadership and Surveillance program (ATLAS) surveillance program [18]. Our study evaluated the distribution of MBL-positive isolates of Enterobacterales collected globally and across different geographical regions, as well as the activity of ATM-AVI and comparators against MBL-positive isolates at global as well as regional levels, with a focus also on NDM-variants and MBL-positive isolates co-carrying other carbapenemases.

2. Methods

2.1. Bacterial isolates

Non-duplicate clinical isolates of Enterobacterales were collected from the participating centres globally between 2016 and 2020 across different regions (Africa and Middle East [AfME], Europe, Asia Pacific [APAC], Latin America [LATAM], and North America) as part of the ATLAS surveillance program. This program collected a predefined set of isolates from each participating centre annually across selected bacterial species and infection types, limiting collection to one isolate per patient every year independent of patient's hospital location [19]. As part of the ATLAS protocol, the participating centres and the number of isolates collected from individual countries or regions varied across the years (Supplemental Table S1). The following species were included: *Escherichia coli, Citrobacter freundii, Citrobacter koseri, Enterobacter asburiae, Enterobacter cloacae, Enterobacter hormaechei, Enterobacter kobei, Enterobacter ludwigii, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, and Serratia marcescens*. Among *E. cloacae* complex [20], *E. nimipressuralis* was excluded as none of the sites on ATLAS program contributed isolates of this species. Isolates from various sources across patients of all ages were identified at each participating centre. These isolates were thereafter shipped to International Health Management Associates, Inc. (IHMA; Schaumburg, IL) for species confirmation using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Biotyper MALDITOF; Bruker Daltonics, Billerica, MA).

2.2. Antimicrobial susceptibility

MICs were evaluated by IHMA using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) [21] and European Committee on Antimicrobial Susceptibility Testing (EUCAST) [22] recommendations. ATM-AVI was compared to the following panel of antimicrobial agents: amikacin, aztreonam (ATM), colistin, gentamicin, meropenem, and tigecycline. The comparator antimicrobial panel selected for this study excluded β-lactam agents that are known to be inactive against MBL-positive isolates, except meropenem [2,23]. Cefiderocol was not included because data was unavailable in the antimicrobial panel on ATLAS during initiation of this study. Of note, not all drugs in the panel were tested every year which resulted in some variation in the total number of isolates tested with each drug.

Susceptibility of antimicrobial agents were interpreted using CLSI M100 (33rd ed.) [24] and EUCAST version 13.0 [22] breakpoints, wherever applicable. ATM-AVI was tested with avibactam at 4 mg/L (fixed concentration). For comparative reasons, a provisional pharmacokinetic/pharmacodynamic breakpoint of $\leq$8 mg/L was considered for the susceptibility of ATM-AVI since there are no defined clinical breakpoints approved [13]. *Proteus mirabilis* and *S. marcescens* were excluded from evaluation of antimicrobial susceptibility to colistin due to their inherent resistance to these antimicrobials [25,26]. *Proteus mirabilis* was also excluded from evaluation of antimicrobial susceptibility to tigecycline for the same reason [25]. Since the CLSI breakpoints for susceptible isolates were not available for colistin, only EUCAST data were reported. For tigecycline, susceptibility rates were determined based on FDA approved breakpoints (in the absence of CLSI breakpoints) [27], and on EUCAST breakpoints for *E. coli* and *C. koseri* only [22].

2.3. Resistance phenotype and genotype definitions

In this study, CRE was defined as isolates of any Enterobacterales species resistant to meropenem per both CLSI and EUCAST breakpoints (i.e. MIC >8 mg/L). Molecular screening by multiplex polymerase chain reaction and sequencing was conducted for all isolates of Enterobacterales with a meropenem MIC >1 mg/L, a ceftazidime/avibactam MIC >8 mg/L (ceftazidime/avibactam resistant), or an ATM-AVI MIC >8 mg/L for the presence of β-lactamase genes [28]. Isolates with the presence of one or more of the major MBL genes (NDM, IMP, VIM [29]) were considered MBL-positive isolates. The current analysis also included MBL-positive isolates co-carrying other carbapenemase genes such as KPC and OXA-48-like. Genotypic variants of NDM genes were considered for subanalysis when the isolate numbers were ≥15.

2.3.1. Statistical methods

Data for Enterobacterales, CRE, and MBL-positive isolates collected between 2016 and 2020 were categorized based on regions (global, AfME, Europe, APAC, LATAM, and North America). The distribution of isolates was determined as percentages of the total number of Enterobacterales, CRE, and MBL-positive isolates collected (global and across various regions). To evaluate significant trends in the percentage of isolates over time, the Cochran Armitage Trend test was used. Only P values of <0.05 were regarded as significant.

3. Results

3.1. Distribution of Enterobacterales and MBL-positive isolates

Overall, a total of 106,686 isolates of Enterobacterales were collected from 1117 sites across 63 countries between 2016 and 2020, globally (Supplementary Table 1). Among all species of Enterobacterales, isolates of E. coli (33.4%) were the most prevalent followed by *K. pneumoniae* (29.4%) and *E. cloacae* (10.6%), with lower numbers of other species (Supplementary Table 2).

Among all Enterobacterales collected globally, CRE isolates were 5.0% and 4.0% per CLSI and EUCAST, respectively. Comparatively higher rates were observed in APAC (CLSI/EUCAST, 8.2%/7.4%) and LATAM (7.1%/5.5%), followed by Europe (4.2%/3.1%) and AfME (3.8%/2.9%), and lowest rate in North America (1.4%/0.7%) (Table 1).

A total of 1707 isolates (1.6%) were MBL-positive, with higher numbers collected from APAC (3.5%), AfME (2.1%), and LATAM (1.9%) (Table 1). The majority of the MBL-positive isolates belonged to *K. pneumoniae* (62.3%), followed by *E. coli* (13.6%) and *E. cloacae* (13.2%) (Supplementary Table 2).

Globally, both CRE and MBL-positive isolates exhibited a significant increasing trend over time ($P < 0.0001$) (Supplementary Table 3). The distribution of MBL-positive isolates showed a significant increasing trend across all regions (AfME, 0.4%–3.4%, $P < 0.0001$; APAC, 1.1%–7.2%, $P < 0.0001$; Europe, 0.5%–1.6%, $P < 0.0001$; LATAM, 0.7%–4.4%, $P < 0.0001$; North America, 0.0%–0.2%, $P = 0.008$) (Supplementary Table 3).

Among MBL-positive isolates, NDM-positive was the most common genotype collected globally (83.3%) and in different regions (59.1%–93.8%) as compared to VIM- and IMP-positive isolates. The majority of NDM-positive isolates (98%) were constituted by NDM-1 (61.4%; 872/1421), NDM-5 (32.4%; 460/1421), and NDM-7 (4.2%; 59/1421). The remaining 2% was constituted by NDM-4, NDM-6, NDM-9, NDM-16, NDM-19, NDM-24, and NDM-TYPE (data not shown). Globally, NDM-1 was the most frequent variant (51.1%) followed by NDM-5 (27%) and NDM-7 (3.5%) (Table 2). Highest frequency of NDM-1 was evident across regions (31.6%–80.6%) except in the APAC region where NDM-5 (55.2%) was predominant (Table 2). With respect to species distribution, the majority of NDM producers belonged to *K. pneumoniae*, among all three NDM variants, globally and across different regions (Supplementary Table 4).

Among the MBL-positive isolates co-carrying other carbapenemases genes, MBL+OXA-48-like isolates were more prevalent than MBL+KPC isolates globally (19.5 % vs. 3.0%) and across different regions (8.9%–37.2% vs. 0.0%–4.1%), except in LATAM (0.7 % vs. 9.2%) (Table 2).

3.2. Antimicrobial activity of ATM-AVI and comparators against Enterobacterales and MBL-positive isolates

ATM-AVI demonstrated potent antimicrobial activity against all Enterobacterales, with MIC_{50} and MIC_{90} values of 0.06 mg/L and 0.25 mg/L, respectively; 99.9% isolates were inhibited at the tentative breakpoint of ≤8 mg/L. Variable susceptibility rates were observed for the comparators with rates >90% for amikacin, tigecycline, meropenem, and colistin (Table 3).

ATM-AVI retained potent antimicrobial activity against MBL-positive isolates, with MIC_{50} and MIC_{90} values of 0.12 and 1 mg/L, respectively; 99.4% of isolates were inhibited at the tentative breakpoint of ≤8 mg/L. Among the comparators, only tigecycline and colistin exhibited susceptibility rates >90% against the MBL-positive isolates (Table 3).

ATM-AVI demonstrated consistent activity against MBL-producing isolates from different regions (98.6% inhibited at the tentative breakpoint of ≤8 mg/L) (Supplementary Table 5).

Table 1
Distribution of Enterobacterales isolates collected globally and across different regions from 2016 to 2020.

Region	Enterobacterales[a] N	CRE (CLSI) n (%)[b]	CRE (EUCAST) n (%)[b]	MBL-Positive[b,c] n (%)
Global	106,686 (100)	5356 (5.0)	4251 (4.0)	1707 (1.6)
AfME	8235 (7.7) (100)	315 (3.8)	241 (2.9)	169 (2.1)
APAC	19,905 (100)	1628 (8.2)	1481 (7.4)	694 (3.5)
Europe	51,115 (100)	2164 (4.2)	1605 (3.1)	539 (1.1)
LATAM	15,244 (100)	1083 (7.1)	835 (5.5)	283 (1.9)
North America	12,187 (100)	166 (1.4)	89 (0.7)	22 (0.2)

AfME, Africa and Middle East; APAC, Asia Pacific; EUCAST, European Committee on Antimicrobial Susceptibility Testing; CLSI, Clinical and Laboratory Standards Institute; CRE, carbapenem-resistant Enterobacterales; LATAM, Latin America; MBL, metallo-β-lactamase; N, total number of Enterobacterales isolates collected globally and in various regions; n, number of CRE and MBL-positive isolates collected globally and in various geographical regions.

[a] *Escherichia coli, Citrobacter freundii, Citrobacter koseri, Enterobacter asburiae, Enterobacter cloacae, Enterobacter hormaechei, Enterobacter kobei, Enterobacter ludwigii, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Serratia marcescens*.

[b] % indicates n/N.

[c] Presence of at least one of the major MBL genes (bla_{NDM}, bla_{VIM}, bla_{IMP}).

Table 2
Distribution of MBL-positive Enterobacterales isolates collected globally and across different regions from 2016 to 2020.

MBL-positive Enterobacterales[a]	Global (*N* = 1707)	AfME (*N* = 169)	APAC (*N* = 694)	Europe (*N* = 539)	LATAM (*N* = 283)	North America (*N* = 22)
VIM, n (%)	242 (14.2)	16 (10.5)	2 (0.3)	197 (36.6)	19 (6.7)	8 (36.4)
IMP, n (%)	49 (2.9)	0 (0.0)	41 (5.9)	2 (0.4)	5 (1.8)	1 (4.6)
NDM, n (%)	1421 (83.3)	153 (90.5)	651 (93.8)	341 (63.3)	263 (92.9)	13 (59.1)
NDM-1, n (%)	872 (51.1)	101 (59.8)	219 (31.6)	313 (58.1)	228 (80.6)	11 (50.0)
NDM-5, n (%)	460 (27.0)	29 (17.2)	383 (55.2)	25 (4.6)	22 (7.8)	1 (4.6)
NDM-7, n (%)	59 (3.5)	21 (12.4)	36 (5.2)	0 (0.0)	1 (0.4)	1 (4.6)
MBL+KPC[b], n (%)	51 (3.0)	0 (0.0)	3 (0.4)	22 (4.1)	26 (9.2)	0 (0.0)
MBL+OXA-48-like[c], n (%)	333 (19.5)	19 (11.2)	258 (37.2)	48 (8.9)	2 (0.7)	6 (27.3)

AfME, Africa and Middle East; APAC, Asia Pacific; LATAM, Latin America; MBL, metallo-β -lactamase; N, total number of isolates; n, number of MBL-positive isolates collected globally and in various geographical regions (major MBL genotypes, NDM variants, and MBL-positive isolates co-carrying other carbapenemases).
[a] % indicates n/N.
[b] KPC-2 and KPC-3.
[c] OXA- 162, OXA-181, OXA-232, and OXA-48.

Table 3
Antimicrobial activity of ATM-AVI and comparator agents tested against Enterobacterales isolates collected globally from 2016 to 2020.

Antimicrobials	n[a]	%S		MIC (mg/L)		
		CLSI	EUCAST[b]	MIC_{50}	MIC_{90}	MIC range
All Enterobacterales[c], *N* = 106,686						
ATM-AVI[d]	82,775		99.9	0.06	0.25	0.015–128
Aztreonam	85,482	70.9	70.9	0.12	128	0.015–>128
Meropenem	106,685	94.4	96.0	0.06	0.12	0.004–32
Amikacin	106,686	96.6	94.7	2	8	0.25–128
Gentamicin	56,648	81.2	80.4	0.5	32	0.12–32
Colistin[e]	77,285	NA	97.1	0.25	1	0.06–16
Tigecycline[f]	102,488	98.2[g]	98.2[h]	0.25	1	0.008–16
MBL-positive Enterobacterales[i], *N* = 1707						
ATM-AVI[d]	1628		99.4	0.12	1	0.015–128
Aztreonam	1693	14.9	14.9	128	256	0.015–>128
Meropenem	1707	3.8	21.7	32	32	0.06–32
Amikacin	1707	50.0	39.5	32	128	0.25–128
Gentamicin	1418	29.0	27.3	32	32	0.12–32
Colistin[e]	1622	NA	91.1	0.5	2	0.06–16
Tigecycline[f]	1674	94.0[g]	92.8[h]	0.5	2	0.06–16

ATM-AVI, aztreonam/avibactam; MBL, metallo-β-lactamase; MIC, minimum inhibitory concentration; MIC_{50} minimum inhibitory concentration required to inhibit 50% of the organisms; MIC_{90} minimum inhibitory concentration required to inhibit 90% of the organisms; N, total number of isolates; n, number of isolates tested every year; NA, not available.
[a] Not all drugs in the panel were tested every year.
[b] Data includes percentage isolates susceptible at increased exposure.
[c] *Escherichia coli, Citrobacter freundii, Citrobacter koseri, Enterobacter asburiae, Enterobacter cloacae, Enterobacter hormaechei, Enterobacter kobei, Enterobacter ludwigii, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Serratia marcescens.*
[d] No breakpoints available from CLSI and EUCAST. Values expressed are indicative of the cumulative percentage of isolates inhibited at ≤8 mg/L of aztreonam, tested at fixed 4 mg/L avibactam concentration, for comparison purposes.
[e] For colistin, CLSI breakpoints for susceptible isolates are not available. EUCAST data for colistin do not include isolates of *P. mirabilis* and *S. marcescens*.
[f] Tigecycline data do not include *P. mirabilis*.
[g] Data for tigecycline were calculated based on FDA approved breakpoints.
[h] EUCAST data for susceptibility to tigecycline are limited to *E. coli* and *C. koseri*; denominator (n): All isolates =38,457, MBL-positive=235.
[i] Presence of at least one of the major MBL genes (bla_{NDM}, bla_{VIM}, bla_{IMP}).

3.3. Antimicrobial activity of ATM-AVI and comparators against various genotypes of MBL-positive isolates

MIC_{90} values of ATM-AVI against different MBL-positive genotypes (NDM, VIM, and IMP) ranged from 0.5 to 1 mg/L, while those of aztreonam were ≥128 mg/L. Among NDM variants, the MIC_{90} of ATM-AVI ranged from 0.5 to 4 mg/L, and those of aztreonam were >128 mg/L. At the tentative breakpoint of ≤8 mg/L', ATM-AVI inhibited 99.8% of isolates producing NDM-1 or NDM-7 (MIC_{90}, 0.5 mg/L), and 98.1% of isolates producing NDM-5 (MIC_{90}, 4 mg/L) (Table 4).

A total of 10 of 1707 MBL-positive isolates (0.6%) exhibited ATM-AVI MICs of >8 mg/L (2019: *n* = 2; 2020: *n* = 8) (Table 4). Of these, 9 were from the APAC region and 1 was from Europe (Supplementary Table 6). All the 10 isolates were NDM-positive; the majority reported as NDM-5 (*n* = 8: *E. coli*, *n* = 7, *K. pneumoniae*, *n* = 1; APAC, *n* = 7, Europe, *n* = 1) and the remaining reported as NDM-1 (*n* = 2: *P. mirabilis*, *n* = 2; APAC, *n* = 2) (Table 4).

For MBL-positive isolates co-carrying OXA-48-like (*N* = 333) or KPC genes (*N* = 51), the MIC_{90} values of ATM-AVI were 0.5 mg/L and 1 mg/L, respectively, as compared to those of aztreonam which were >128 mg/L. Among these, only 1/384 of the co-carriers had ATM-AVI MIC >8 mg/L (Table 4).

Among comparators, tigecycline and colistin showed an overall high antimicrobial activity (≥80% susceptibility) against various MBL-positive genotypes, with some variability observed. Additionally, higher susceptibility to amikacin was observed among NDM-7-positive, VIM-positive, and IMP-positive isolates (Table 5).

Table 4
Percentage MIC (mg/L) frequency distribution for Enterobacterales collected globally from 2016 to 2020.

Organism/organism group	Antimicrobial	N	Cumulative percentage of isolates at each MIC (mg/L) (absolute n at MIC)														
			≤0.015	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**
All Enterobacterales[a]	ATM-AVI	106,686	16.5 (13,687)	47.1 (25,320)	76.9 (24,667)	89.8 (10,689)	**95.4** (**4599**)	97.8 (2029)	98.9 (841)	99.5 (512)	99.8 (288)	99.9 (70)	99.9 (39)	99.9 (9)	99.9 (14)	100.0 (11)	
	ATM		4.6 (3905)	14.4 (8392)	39.9 (21,764)	58.5 (15,906)	64.6 (5250)	66.7 (1821)	68.1 (1175)	69.3 (1050)	70.9 (1364)	73.4 (2153)	77.1 (3131)	83.5 (5472)	89.6 (5210)	**96.0** **(5487)**	100.0 (3402)
MBL-positive Enterobacterales	ATM-AVI	1707	2.6 (42)	8.1 (90)	23.5 (250)	51.5 (456)	77.3 (420)	87.8 (171)	**92.2** **(72)**	95.8 (59)	98.4 (42)	99.4 (16)	99.8 (7)	99.9 (1)	99.9 (0)	100.0 (2)	
	ATM		0.3 (5)	1.0 (11)	2.6 (28)	5.6 (50)	8.5 (49)	10.9 (42)	11.8 (15)	13.4 (26)	14.9 (27)	17.5 (43)	20.7 (54)	27.4 (113)	42.7 (259)	83.6 (694)	**100.0** **(277)**
VIM	ATM-AVI	242	3.8 (9)	15.0 (27)	38.3 (56)	57.1 (45)	74.2 (41)	89.2 (36)	**98.3** **(22)**	100.0 (4)							
	ATM		0.0 (0)	2.1 (5)	6.7 (11)	12.1 (13)	15.8 (9)	19.2 (8)	19.6 (1)	24.2 (11)	28.3 (10)	34.6 (15)	40.8 (15)	48.8 (19)	70.4 (52)	**92.5** **(53)**	100.0 (18)
IMP	ATM-AVI	49	2.1 (1)	6.4 (2)	29.8 (11)	59.6 (14)	85.1 (12)	**93.6** **(4)**	93.6 (0)	100.0 (3)							
	ATM		0.0 (0)	0.0 (0)	8.5 (4)	12.8 (2)	23.4 (5)	25.5 (1)	27.7 (1)	34.0 (3)	40.4 (3)	44.7 (2)	53.2 (4)	61.7 (4)	80.9 (9)	**93.6** **(6)**	100.0 (3)
NDM	ATM-AVI	1421	2.4 (32)	7.0 (62)	20.7 (185)	50.2 (397)	77.6 (368)	87.4 (132)	**91.1** **(50)**	95.0 (52)	98.1 (42)	99.3 (16)	99.8 (7)	99.9 (1)	99.9 (0)	100.0 (2)	
	ATM		0.4 (5)	0.8 (6)	1.7 (13)	4.2 (35)	6.7 (35)	9.1 (34)	10.0 (13)	10.8 (12)	12.1 (17)	13.9 (26)	16.4 (35)	22.8 (90)	36.9 (199)	81.9 (635)	**100.0** **(256)**
NDM-1	ATM-AVI	872	2.9 (24)	8.6 (48)	26.0 (146)	63.7 (317)	87.9 (203)	**96.6** **(73)**	98.2 (14)	99.2 (8)	99.6 (4)	99.8 (1)	100.0 (2)				
	ATM		0.5 (4)	0.9 (4)	2.2 (11)	5.6 (29)	9.2 (31)	11.5 (20)	12.0 (4)	12.9 (8)	14.4 (13)	16.8 (21)	19.4 (22)	25.8 (55)	41.2 (133)	84.1 (370)	**100.0** **(137)**
NDM-5	ATM-AVI	460	1.2 (5)	3.1 (8)	8.3 (22)	21.0 (53)	55.2 (144)	67.1 (50)	75.5 (35)	86.0 (44)	**95.0** **(38)**	98.1 (13)	99.3 (5)	99.5 (1)	99.5 (0)	100.0 (2)	
	ATM		0.2 (1)	0.2 (0)	0.4 (1)	0.7 (1)	1.5 (4)	3.7 (10)	5.7 (9)	6.5 (4)	7.0 (2)	8.0 (5)	10.2 (10)	16.3 (28)	28.0 (54)	77.8 (229)	**100.0** **(102)**
NDM-7	ATM-AVI	59	0.0 (0)	7.0 (4)	26.3 (11)	59.7 (19)	86.0 (15)	**96.5** **(6)**	96.5 (0)	96.5 (0)	96.5 (0)	100.0 (2)					
	ATM			1.7 (1)	3.4 (1)	8.5 (3)	8.5 (0)	13.6 (3)	13.6 (0)	13.6 (0)	17.0 (2)	17.0 (0)	18.7 (1)	25.4 (4)	39.0 (8)	76.3 (22)	**100.0** **(14)**
MBL + KPC[b]	ATM-AVI	51	0.0 (0)	8.0 (4)	26.0 (9)	54.0 (14)	70.0 (8)	86.0 (8)	**100.0** **(7)**								
	ATM		0.0 (0)	0.0 (0)	0.0 (0)	2.0 (1)	2.0 (0)	2.0 (0)	2.0 (0)	2.0 (0)	6.0 (2)	6.0 (0)	10.0 (2)	16.0 (3)	28.0 (6)	84.0 (28)	**100.0** **(8)**
MBL + OXA-48-like[c]	ATM-AVI	333	0.3 (1)	1.3 (3)	4.6 (10)	20.9 (50)	72.9 (159)	**91.8** **(58)**	94.4 (8)	96.4 (6)	99.4 (9)	99.7 (1)	99.7 (0)	99.7 (0)	99.7 (0)	100.0 (1)	
	ATM		0.0 (0)	0.3 (1)	0.6 (1)	0.9 (1)	1.8 (3)	5.4 (12)	6.3 (3)	6.6 (1)	6.9 (1)	7.8 (3)	8.7 (3)	11.2 (8)	22.3 (37)	75.0 (175)	**100.0** **(83)**

ATM, aztreonam; ATM-AVI, aztreonam/avibactam; MBL, metallo-β-lactamase; N, total number of isolates.

[a] *Escherichia coli, Citrobacter freundii, Citrobacter koseri, Enterobacter asburiae, Enterobacter cloacae, Enterobacter hormaechei, Enterobacter kobei, Enterobacter ludwigii, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Serratia marcescens*.

[b] KPC-2 and KPC-3.

[c] OXA- 162, OXA-181, OXA-232, and OXA-48.

MIC_{90} values are in bold.

4. Discussion

Our study highlighted the distribution of MBL-positive Enterobacterales collected globally and across different regions over a five-year period (2016–2020) from the ATLAS surveillance system [18], and the antimicrobial activity of ATM-AVI and comparator agents tested against these isolates. To the best of our knowledge, this is the largest collection of isolates investigated so far for ATM-AVI studies.

Globally, our study identified 4.0/5.0% (CLSI/EUCAST criteria) CRE and 1.6% MBL-positive isolates among total Enterobacterales collected. The MBL-positive isolates were notably higher than those

Table 5
Antimicrobial activity of ATM-AVI and comparator agents tested against MBL-positive Enterobacterales isolates collected globally from 2016 to 2020.

Antimicrobials	n[a]	%S		MIC (mg/L)		
		CLSI	EUCAST[b]	MIC_{50}	MIC_{90}	MIC range
VIM, *N* = 242						
ATM-AVI[c]	240	100		0.12	1	0.015–2
Aztreonam	240	28.3	28.3	64	128	0.03–>128
Meropenem	242	16.9	65.3	8	32	0.06–32
Amikacin	242	88.4	72.3	8	32	0.25–128
Gentamicin	168	60.7	52.4	2	32	0.5–32
Colistin[d]	224	NA	93.3	0.5	1	0.06–16
Tigecycline[e]	237	91.1[f]	100[g]	0.5	2	0.12–16
IMP, *N* = 49						
ATM-AVI[c]	47	100		0.12	0.5	0.015–2
Aztreonam	47	40.4	40.4	16	128	0.06–>128
Meropenem	49	28.6	87.8	4	16	0.25–32
Amikacin	49	87.8	81.6	2	64	0.5–128
Gentamicin	17	35.3	23.5	32	32	0.5–32
Colistin[d]	45	NA	100	0.25	0.5	0.12–2
Tigecycline[e]	49	93.9[f]	100[g]	0.5	2	0.12–4
NDM, *N* = 1421						
ATM-AVI[c]	1346	99.3		0.12	1	0.015–128
Aztreonam	1411	12.1	12.1	128	256	0.015–>128
Meropenem	1421	0.7	12.0	32	32	0.06–32
Amikacin	1421	41.9	32.3	64	128	0.25–128
Gentamicin	1238	24.7	24.1	32	32	0.12–32
Colistin[d]	1357	NA	90.5	0.5	2	0.06–16
Tigecycline[e]	1393	94.5[f]	92.5[g]	0.5	2	0.06–16
NDM-1, *N* = 872						
ATM-AVI[c]	840	99.8		0.12	0.5	0.015–16
Aztreonam	862	14.4	14.4	128	256	0.015–>128
Meropenem	872	0.6	16.6	32	32	0.06–32
Amikacin	872	45.2	32.2	32	128	0.5–128
Gentamicin	725	28.0	26.9	32	32	0.12–32
Colistin[d]	815	NA	88.1	0.5	8	0.06–16
Tigecycline[e]	848	93.0[f]	88.9[g]	0.5	2	0.06–16
NDM-5, *N* = 460						
ATM-AVI[c]	420	98.1		0.25	4	0.015–128
Aztreonam	460	7.0	7.0	128	256	0.015–>128
Meropenem	460	0.7	4.1	32	32	0.06–32
Amikacin	460	27.2	24.1	128	128	0.25–>128
Gentamicin	446	16.8	16.8	32	32	0.12–32
Colistin[d]	457	NA	93.7	0.25	1	0.12–16
Tigecycline[e]	459	97.0[f]	93.9[g]	0.5	2	0.06–16
NDM-7, *N* = 59						
ATM-AVI[c]	57	100		0.12	0.5	0.03–8
Aztreonam	59	17.0	17.0	128	256	0.03–>128
Meropenem	59	1.7	5.1	32	32	0.06–32
Amikacin	59	94.9	84.8	4	16	1–64
Gentamicin	38	39.5	39.5	32	32	0.25–32
Colistin[d]	58	NA	96.6	0.5	1	0.06–16
Tigecycline[e]	59	94.9[f]	66.7[g]	0.5	2	0.12–16
MBL+KPC[h], *N* = 51						
ATM-AVI[c]	50	100		0.12	1	0.03–1
Aztreonam	50	6.0	6.0	128	256	0.12–>128
Meropenem	51	0	7.8	32	32	2–32
Amikacin	51	60.8	54.9	8	128	1–128
Gentamicin	47	27.7	23.4	32	32	0.25–32
Colistin[d]	50	NA	80.0	0.5	16	0.06–16
Tigecycline[e]	51	92.2[f]	100[g]	0.5	2	0.12–16
MBL+OXA-48-like[i], *N* = 333						
ATM-AVI[c]	306	99.7		0.25	0.5	0.015–128
Aztreonam	332	6.9	6.9	128	256	0.03–>128
Meropenem	333	0.6	3.9	32	32	0.5–32
Amikacin	333	19.5	15.3	128	128	0.25–128
Gentamicin	310	12.9	12.6	32	32	0.12–32
Colistin[d]	330	NA	86.1	0.5	8	0.06–16

(*continued on next page*)

Table 5 (*continued*)

Antimicrobials	n[a]	%S		MIC (mg/L)		
		CLSI	EUCAST[b]	MIC_{50}	MIC_{90}	MIC range
Tigecycline[e]	332	95.5[f]	95.7[g]	1	2	0.06–4

ATM-AVI, aztreonam/avibactam; MBL, metallo-β-lactamase; MIC, minimum inhibitory concentration; MIC_{50} minimum inhibitory concentration required to inhibit 50% of the organisms; MIC_{90} minimum inhibitory concentration required to inhibit 90% of the organisms; N, total number of isolates; n, number of isolates tested every year; NA, not available.

[a] Not all drugs in the panel were tested every year.

[b] Data includes percentage isolates susceptible at increased exposure.

[c] No breakpoints available from CLSI and EUCAST. Values expressed are indicative of the cumulative percentage of isolates inhibited at ≤8 mg/L of aztreonam, tested at fixed 4 mg/L avibactam concentration, for comparison purposes.

[d] For colistin, CLSI breakpoints for susceptible isolates are not available. EUCAST data for colistin do not include isolates of *P. mirabilis* and *S. marcescens*.

[e] Tigecycline data do not include *P. mirabilis*.

[f] Data for tigecycline were calculated based on FDA approved breakpoints.

[g] EUCAST data for susceptibility to tigecycline are limited to *E. coli* and *C. koseri*; denominator (n): NDM=227; VIM=8; IMP=1; NDM-1 = 36; NDM-5 = 181; NDM-7 = 6; MBL+KPC=3;MBL+OXA-48-like=23.

[h] KPC-2 and KPC-3.

[i] OXA- 162, OXA-181, OXA-232, and OXA-48.

reported previously from global surveillance studies by Karlowsky et al. (AstraZeneca surveillance program; 208 medical centres in 40 countries, 2012–2015) [15] and by Kazmierczak et al. (202 medical centres in 40 countries, 2012–2014) [30], which reported MBL rates of 0.5% (267/51,352) and 0.4% (163/38,266), respectively. In our study, a significant increasing trend of MBL-positive isolates was identified globally and across all regions. A recent study trend (INFORM surveillance program, 2019–2021) conducted in the United States also reported an increase in MBL-positive isolates over the years (2019, 3/9686 [0.03%]; 2021, 20/8916 [0.2%]) [31]. Notably, the highest proportion of MBL-positive isolates in our study was contributed by the APAC region (3.5%). These findings were corroborated by another global study by Sader et al. [16] (SENTRY surveillance program; 64 medical centers in Europe, APAC, and LATAM) conducted in 2019 (overall MBLs, 1.5%, 132/8787; APAC, 3.2%, 47/1456; Europe, 1.2%, 73/6170). However, contrary to these findings, Karlowsky et al. [15] reported a lower proportion of MBLs during 2012–2015 (overall MBLS, 0.5%, 267/51,352; APAC, 0.8%, 74/9149; Europe, 0.5%, 131/24,826). Therefore, our study implies a longitudinal increase in MBL incidence by year. Recent data from a regional multicentre study conducted in 8 APAC countries (2015–2018) showed an even higher proportion of MBL-positive isolates (8.5%, 71/832) among Enterobacterales collected [32], compared to our study (3.5%, 694/19,905). Taken together, these findings suggest a variation in the distribution of MBL-positive isolates across the globe underscoring the need for reinforcing surveillance both at global and regional levels, and for implementation of robust infection control. This also warrants an imperative need for therapeutic strategies that may be critical in limiting further spread.

Among the MBL-positive isolates in our study, NDM-positive isolates were the most common (83.3%) with NDM-1 isolates as the majority (61.4% of NDMs), especially in Europe (NDM/NDM-1, 63.3%/91.8% [313/341]). This was consistent with the recently published studies from the SENTRY database: 2019 (global): 87.1% /78.3% [16]; 2019–2020 (Europe): 77.1% /96.4% [14]. Previous studies also reported a higher frequency of NDM-positive isolates (global studies: 2012–2015, 53.18%, 142/267 [15]; 2012–2014, 44.2%, 72/163 [30]). In contrast, a global study by Biedenbach et al. [33] showed majority of VIM-positive isolates (66.5%, 141/212) with only 16.0% (34/212) NDM-positive isolates among Enterobacterales, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* collected between 2012 and 2013. Additionally, our study identified that NDM-5 was the predominant NDM in the APAC region (55.2%). The emergence and spread of NDM-5 in the APAC region are supported by other studies and possibly attributed to dissemination mechanism as well as nosocomial transmission [34]. Therefore, these findings suggest a global spread of NDM-positive isolates and variations in NDM-positive subtypes across regions over the last decade.

Our study demonstrated MBL-positive isolates co-carrying OXA-48-like genes (19.5%) and KPC genes (3.0%). Similar findings were reported by a global SENTRY study (2019, MBL+OXA-48-like, 14.4% [19/132] and MBL+KPC, 3.0% [4/132]) [16], and by Karlowsky et al. [15] (2012–2015, MBL+OXA-48-like, 11.2% [30/267] and MBL+KPC, 3.0% [8/267]). This suggests an increase of MBL+OXA-48-like isolates over the years, which is a serious concern with the current scenario of limited treatment options.

Overall, ATM-AVI demonstrated a potent antimicrobial activity against all Enterobacterales (MIC_{90}, 0.25 mg/L) and MBL-positive isolates (MIC_{90}, 1 mg/L) in comparison to aztreonam (MIC_{90}, ≥128 mg/L). At the tentative breakpoint of ≤8 mg/L, ATM/AVI was able to inhibit 99.9% of Enterobacterales and 99.4% of MBL-positive Enterobacterales, respectively. These findings are consistent with those from the recent global SENTRY study (2019) showing 99.9% of Enterobacterales (MIC_{90}, 0.12 mg/L) and all MBL-positive isolates (MIC_{90}, 0.5 mg/L) inhibited at MIC ≤8 mg/L for ATM-AVI [16]. This was further corroborated by another global study conducted between 2012 and 2015 (MIC_{90}: Enterobacterales, 0.12 mg/L; MBL-positive isolates, 1 mg/L) [15]. Additionally, although the global activity of ATM-AVI was sustained across regions in our study, the MIC values against MBL-positive isolates in the APAC region (MIC_{90}, 2 mg/L) were higher compared to those observed with isolates from other regions (MIC_{90}, 0.25 mg/L in LATAM; MIC_{90}, 0.5 mg/L in other regions). A previous study by Karlowsky et al. [15] (2012–2015) showed an MIC_{90} of 0.5 mg/L in APAC. In our study, ATM-AVI inhibited 98.1% of the NDM-5 isolates at MIC of ≤8 mg/L and had an MIC_{90} value of 4 mg/L, which was almost 3–4 dilutions higher than the other isolates tested. The higher MIC values observed in the APAC region could be related with higher prevalence of isolates producing NDM-5, which showed higher MIC values. Altogether, these results support the sustained in vitro activity of ATM-AVI against MBL-positive isolates over the recent years, although increasing MIC levels of ATM-AVI against some isolates must be monitored closely.

Our study also demonstrated high potency of ATM-AVI (99.7% at the tentative breakpoint of ≤8 mg/L) against MBL-positive isolates co-carrying either KPC or OXA-48-like genes (MIC_{90}, 0.5–1 mg/L vs. aztreonam MIC_{90}, >128 mg/L). This is corroborated by global studies conducted previously [15,33,35]. In our study, isolates carrying triple carbapenemase genes (MBL+KPC+OXA-48-like) were not identified.

Our study identified 10 MBL-positive isolates with MIC >8 mg/L for ATM-AVI, with majority of them from the APAC region and har-

bouring NDM-5. A previous study by Vasoo et al. [36] reported an NDM-7-positive isolate from Singapore with elevated MIC of 16 mg/L for ATM-AVI among 177 Gram-negative isolates collected across United States, Singapore, and United Kingdom. A recent study by Livermore et al. [37] conducted in the United Kingdom (2015, 2017, 2019) found 14.8% of NDM-positive *E. coli* isolates (18/122) with MIC of ATM-AVI >8 mg/L and 47.2% NDM-5 isolates (n = 42) among 89 *E.coli* NDM-positive isolates with MIC between 8 and 32 mg/L. Elevated MIC of ATM-AVI may be due to various potential resistance mechanisms including alteration in penicillin-binding protein-3 target [38], presence of some AmpC-type β-lactamases [37], loss of outer membrane proteins [39], and overexpression of efflux pumps [36].

Our study has some limitations. The inherent design of the ATLAS program with the majority of centres from Europe (n = 533) compared to other regions (AfME, n = 87) could lead to bias in data interpretation. Additionally, variations in the number of participating centres and of collected isolates in different years from different regions (including those with high MBL prevalence) may restrict generalisation of results and should be considered while interpreting the study data. Due to the predefined number of isolates collected by each centre, the study results may not be interpreted as general epidemiological data. Further, sample size for a few geographical regions were low for conclusive evidence. Additionally, the 2023 version for CLSI and EUCAST were used for interpretation of antimicrobial susceptibility testing results, which should be taken into consideration while interpreting susceptibility data based on any other clinical breakpoint systems (e.g. the United States Committee on Antimicrobial Susceptibility Testing [40]). This study investigated the major MBL genotypes and did not include other MBL genes such as SPM, GIM, and FIM [29]. Since the study focuses on MBL active agents, other antimicrobials known to be inactive against MBL-positive isolates were excluded [2]. Due to unavailability of data in the ATLAS platform, the following datasets could not be captured and were not reported in this paper: (i) data analysis for MBL isolates that exclusively harboured the dual genotypes (e.g. a NDM+VIM isolate were included in NDM or VIM without a separate category for dual genotype); (ii) susceptibility to some antimicrobials (e.g. fosfomycin, cefiderocol) [2]; and (iii) data analysis based on the type of MBL-positive isolates co-carrying other carbapenemases (NDM/VIM/IMP isolates co-carrying KPC or OXA-48-like genes). Furthermore, this study did not molecularly characterize the isolates with elevated ATM-AVI MICs (>8 mg/L) and elucidate the underlying mechanism(s).

To conclude, this study demonstrated potent antimicrobial activity of ATM-AVI against MBL-positive isolates (including MBL subtypes and variants of NDM-positive isolates) globally and across various regions between 2016 and 2020. Moreover, sustained in vitro activity was observed with those MBL-positive isolates co-carrying other carbapenemases genes (KPC and OXA-48-like). With limited therapeutic options available at present, the combination of aztreonam and avibactam (phase 3 trials completed, NCT03580044, NCT03329092, both results not yet published; paediatric trial ongoing, NCT05639647) may represent a good option for treating infections caused by MBL-positive isolates.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.12.027.

References

[1] Wangchinda W, Thamlikitkul V, Watcharasuwanseree S, Tangkoskul T. Active surveillance for carbapenem-resistant Enterobacterales (CRE) colonization and clinical course of CRE colonization among hospitalized patients at a University Hospital in Thailand. Antibiotics (Basel) 2022;11:1401. doi:10.3390/antibiotics11101401.
[2] Tan X, Kim HS, Baugh K, Huang Y, Kadiyala N, Wences M, et al. Therapeutic options for metallo-beta-lactamase-producing Enterobacterales. Infect Drug Resist 2021;14:125–42. doi:10.2147/IDR.S246174.
[3] Hardiman CA, Weingarten RA, Conlan S, Khil P, Dekker JP, Mathers AJ, et al. Horizontal transfer of carbapenemase-encoding plasmids and comparison with hospital epidemiology data. Antimicrob Agents Chemother 2016;60:4910–19. doi:10.1128/AAC.00014-16.
[4] Tesfa T, Mitiku H, Edae M, Assefa N. Prevalence and incidence of carbapenem-resistant *K. pneumoniae* colonization: systematic review and meta-analysis. Syst Rev 2022;11:240. doi:10.1186/s13643-022-02110-3.
[5] Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. Deaths attributable to carbapenem-resistant *Enterobacteriaceae* infections. Emerg Infect Dis 2014;20:1170–5. doi:10.3201/eid2007.121004.
[6] van Duin D, Doi Y. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. Virulence 2017;8:460–9. doi:10.1080/21505594.2016.1222343.
[7] Walsh TR. Emerging carbapenemases: a global perspective. Int J Antimicrob Agents 2010;36(Suppl 3):S8–14. doi:10.1016/S0924-8579(10)70004-2.
[8] Suay-Garcia B, Perez-Gracia MT. Present and future of carbapenem-resistant *Enterobacteriaceae* (CRE) infections. Antibiotics (Basel) 2019;8:122. doi:10.3390/antibiotics8030122.
[9] Islam K, Heffernan AJ, Naicker S, Henderson A, Chowdhury MAH, Roberts JA, et al. Epidemiology of extended-spectrum beta-lactamase and metallo-beta-lactamase-producing *Escherichia coli* in South Asia. Future Microbiol 2021;16:521–35. doi:10.2217/fmb-2020-0193.
[10] Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? Clin Microbiol Rev 2005;18:306–25. doi:10.1128/CMR.18.2.306-325.2005.
[11] van Duin D, Kaye KS, Neuner EA, Bonomo RA. Carbapenem-resistant *Enterobacteriaceae*: a review of treatment and outcomes. Diagn Microbiol Infect Dis 2013;75:115–20. doi:10.1016/j.diagmicrobio.2012.11.009.
[12] Ayoub Moubareck C. Polymyxins and bacterial membranes: a review of antibacterial activity and mechanisms of resistance. Membranes (Basel) 2020;10:181. doi:10.3390/membranes10080181.
[13] Rossolini GM, Stone G, Kantecki M, Arhin FF. In vitro activity of aztreonam/avibactam against isolates of Enterobacterales collected globally from ATLAS in 2019. J Glob Antimicrob Resist 2022;30:214–21. doi:10.1016/j.jgar.2022.06.018.
[14] Sader HS, Mendes RE, Arends SJR, Carvalhaes CG, Castanheira M. 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. doi:10.1007/s10096-022-04400-z.
[15] Karlowsky JA, Kazmierczak KM, de Jonge BLM, Hackel MA, Sahm DF, Bradford PA. 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. doi:10.1128/AAC.00472-17.
[16] Sader HS, Carvalhaes CG, Arends SJR, Castanheira M, Mendes RE. Aztreonam/avibactam activity against clinical isolates of Enterobacterales collected in Europe, Asia and Latin America in 2019. J Antimicrob Chemother 2021;76:659–66. doi:10.1093/jac/dkaa504.

[17] Sader HS, Mendes RE, Pfaller MA, Shortridge D, Flamm RK, Castanheira M. Antimicrobial activities of aztreonam-avibactam and comparator agents against contemporary (2016) clinical *Enterobacteriaceae* isolates. Antimicrob Agents Chemother 2018;62 e01856–17. doi:10.1128/AAC.01856-17.
[18] Antimicrobial Testing Leadership and Surveillance (ATLAS). https://atlas-surveillance.com/#/login [accessed December 23, 2022].
[19] Karlowsky JA, Bouchillon SK, Benaouda A, Soraa N, Zerouali K, Mohamed N, et al. Antimicrobial susceptibility testing of clinical isolates of Gram-negative bacilli collected in Morocco by the ATLAS Global Surveillance Program from 2018 to 2020. J Glob Antimicrob Resist 2022;30:23–30. doi:10.1016/j.jgar.2022.04.011.
[20] Mezzatesta ML, Gona F, Stefani S. *Enterobacter cloacae* complex: clinical impact and emerging antibiotic resistance. Future Microbiol 2012;7:887–902. doi:10.2217/fmb.12.61.
[21] Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 11th ed. CLSI standard M07. Wayne, PA: CLSI; 2018. https://clsi.org/media/1928/m07ed11_sample.pdf [accessed December 6, 2022].
[22] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.0. 2023. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.0_Breakpoint_Tables.pdf [accessed January 11, 2023].
[23] Armstrong T, Fenn SJ, Hardie KR. JMM Profile: carbapenems: a broad-spectrum antibiotic. J Med Microbiol 2021;70:001462. doi:10.1099/jmm.0.001462.
[24] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testingCLSI supplement M100. 33rd ed. CLSI; 2023 https://clsi.org/standards/products/microbiology/documents/m100/ [accessed May 11, 2023].
[25] Gogry FA, Siddiqui MT, Sultan I, Haq QMR. Current update on intrinsic and acquired colistin resistance mechanisms in bacteria. Front Med (Lausanne) 2021;8:677720. doi:10.3389/fmed.2021.677720.
[26] Qin S, Qi H, Zhang Q, Zhao D, Liu ZZ, Tian H, et al. Emergence of extensively drug-resistant *Proteus mirabilis* harbouring a conjugative NDM-1 plasmid and a novel Salmonella Genomic Island 1 Variant, SGI1-Z. Antimicrob Agents Chemother 2015;59:6601–4. doi:10.1128/AAC.00292-15.
[27] Pfizer, Inc. Tygacil (tigecycline) prescribing information. 2021. https://labeling.pfizer.com/ShowLabeling.aspx?format=PDF&id=491 [accessed December 6, 2022].
[28] Karlowsky JA, Bouchillon SK, El Mahdy Kotb R, Mohamed N, Stone GG, Sahm DF. Carbapenem-resistant Enterobacterales and *Pseudomonas aeruginosa* causing infection in Africa and the Middle East: a surveillance study from the ATLAS programme (2018-20). JAC Antimicrob Resist 2022;4:dlac060. doi:10.1093/jacamr/dlac060.
[29] Sawa T, Kooguchi K, Moriyama K. Molecular diversity of extended-spectrum beta-lactamases and carbapenemases, and antimicrobial resistance. J Intensive Care 2020;8:13. doi:10.1186/s40560-020-0429-6.
[30] Kazmierczak KM, Rabine S, Hackel M, McLaughlin RE, Biedenbach DJ, Bouchillon SK, et al. Multiyear, multinational survey of the incidence and global distribution of metallo-beta-lactamase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2016;60:1067–78. doi:10.1128/AAC.02379-15.
[31] Sader HS, Mendes RE, Carvalhaes CG, Kimbrough JH, Castanheira M. Changing epidemiology of carbapenemases among carbapenem-resistant enterobacterales from United States hospitals and the activity of aztreonam-avibactam against contemporary enterobacterales (2019-2021). Open Forum Infect Dis 2023;10:ofad046. doi:10.1093/ofid/ofad046.
[32] Chen YC, Chen WY, Hsu WY, Tang HJ, Chou Y, Chang YH, et al. Distribution of beta-lactamases and emergence of carbapenemases co-occurring Enterobacterales isolates with high-level antibiotic resistance identified from patients with intra-abdominal infection in the Asia-Pacific region, 2015-2018. J Microbiol Immunol Infect 2022;55:1263–72. doi:10.1016/j.jmii.2021.07.007.
[33] Biedenbach DJ, Kazmierczak K, Bouchillon SK, Sahm DF, Bradford PA. In vitro activity of aztreonam-avibactam against a global collection of Gram-negative pathogens from 2012 to 2013. Antimicrob Agents Chemother 2015;59:4239–48. doi:10.1128/AAC.00206-15.
[34] Li J, Yu T, Tao XY, Hu YM, Wang HC, Liu JL, et al. Emergence of an NDM-5-producing *Escherichia coli* sequence type 410 clone in infants in a children's hospital in China. Infect Drug Resist 2020;13:703–10. doi:10.2147/IDR.S244874.
[35] Kazmierczak KM, Biedenbach DJ, Hackel M, Rabine S, de Jonge BL, Bouchillon SK, et al. Global dissemination of blaKPC into bacterial species beyond *Klebsiella pneumoniae* and in vitro susceptibility to ceftazidime-avibactam and aztreonam-avibactam. Antimicrob Agents Chemother 2016;60:4490–500. doi:10.1128/AAC.00107-16.
[36] Vasoo S, Cunningham SA, Cole NC, Kohner PC, Menon SR, Krause KM, et al. In vitro activities of ceftazidime-avibactam, aztreonam-avibactam, and a panel of older and contemporary antimicrobial agents against carbapenemase-producing gram-negative bacilli. Antimicrob Agents Chemother 2015;59:7842–6. doi:10.1128/AAC.02019-15.
[37] Livermore DM, Mushtaq S, Vickers A, Woodford N. Activity of aztreonam/avibactam against metallo-β-Lactamase-producing Enterobacterales from the UK: impact of penicillin-binding protein-3 inserts and CMY-42 β-lactamase in *Escherichia coli*. Int J Antimicrob Agents 2023;61:106776. doi:10.1016/j.ijantimicag.2023.106776.
[38] Alm RA, Johnstone MR, Lahiri SD. Characterization of *Escherichia coli* NDM isolates with decreased susceptibility to aztreonam/avibactam: role of a novel insertion in PBP3. J Antimicrob Chemother 2015;70:1420–8. doi:10.1093/jac/dku568.
[39] Li H, Estabrook M, Jacoby GA, Nichols WW, Testa RT, Bush K. In vitro susceptibility of characterized beta-lactamase-producing strains tested with avibactam combinations. Antimicrob Agents Chemother 2015;59:1789–93. doi:10.1128/AAC.04191-14.
[40] The United States Committee on Antimicrobial Susceptibility Testing. https://www.uscast.org/ [accessed November 1, 2023].