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In vitro activity of aztreonam-avibactam (ATM-AVI) against Gram-negative pathogens from Europe collected in 2014

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Revised Abstract

Background: Avibactam (AVI) is a non-β-lactam β-lactamase inhibitor with activity against class A, class B, and some class D β-lactamases, including extended-spectrum β-lactamases (ESBLs) and KPCs. Aztreonam (ATM) is stable to hydrolysis by metallo-β-lactamases (MBL). ATM-AVI is being developed for use against carbapenem-resistant *Enterobacteriaceae*, especially those producing MBLs that often co-carry serine β-lactamases. This study evaluated the *in vitro* activity of ATM-AVI and comparators against *Enterobacteriaceae* and *P. aeruginosa* collected in 2014 in Europe. **Methods:** Non-duplicate clinical isolates were collected from 78 centers in 18 European countries. Susceptibility testing was performed using CLSI broth microdilution and interpreted using EUCAST 2015 breakpoints. Aztreonam-avibactam was tested at a fixed concentration of 4 mg/L avibactam. PCR and sequencing were used to determine the β-lactamase genes present in isolates that were non-susceptible to carbapenems (meropenem, imipenem, doripenem), phenotypically positive for ESBL, and those with ceftazidime MICs ≥ 16 mg/L. **Results:** The MIC data for *Enterobacteriaceae* and *P. aeruginosa* are provided in the table. ATM-AVI demonstrated good activity against *Enterobacteriaceae*, with MIC_{90} values of 0.12-1 mg/L for all groups. 99.9% of the isolates, including those that produced MBLs, were inhibited by ≤ 8 mg/L of ATM-AVI. VIM- and NDM-type MBLs were found in 17 *K. pneumoniae*, 7 *E. cloacae*, 3 *C. freundii*, 3 *S. marcescens*, 2 *P. mirabilis*, and 1 *P. stuartii* collected in Greece, Romania, Hungary, Russia, Italy, Turkey, Germany and the United Kingdom. No IMP-type MBLs were found in *Enterobacteriaceae* isolates from Europe. All MBL-producing *Enterobacteriaceae* isolates co-carried one or more plasmid- or chromosomally-mediated serine β-lactamases, including CTX-M-15 and OXA-48. ATM-AVI showed only modest activity against *P. aeruginosa*.

	Drug (MIC_{50} [mg/L], % Susceptible)								
Species/ phenotype[a] (n)	ATM-AVI		ATM		MEM		COL		TGC
Enterobacteriaceae All (7453)	0.12	NA	64	74.1%	0.12	97.3%	>4	82.7%	2	84.6%
ESBL-positive (1367)	0.25	NA	>128	1.4%	2	91.1%	2	91.7%	2	83.6%
Meropenem-S (7250)	0.12	NA	64	76.1%	0.12	100%	>4	83.0%	2	84.9%
Meropenem-NS (203)	0.5	NA	>128	4.4%	>8	0	>4	72.4%	2	70.9%
MBL-negative (7420)	0.12	NA	64	74.4%	0.12	97.6%	>4	82.7%	2	84.6%
MBL-positive (33)	1	NA	>128	21.2%	>8	21.2%	>4	78.8%	2	72.7%
P. aeruginosa All (2091)	32	NA	32	4.4%	>8	72.5%	2	99.6%	>8	NA

ATM-AVI, aztreonam-avibactam; ATM, aztreonam; MEM, meropenem; COL, colistin; TGC, tigecycline; NA, no breakpoints available; NS, non-susceptible
[a]ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamase.

Conclusions: ATM-AVI had good activity against *Enterobacteriaceae* isolated in Europe, including those that produced ESBLs and MBLs. ATM-AVI was highly active against all MBL-containing *Enterobacteriaceae*, regardless of species or country of isolation. The promising *in vitro* activity of ATM-AVI against carbapenem-resistant *Enterobacteriaceae*, especially those producing MBLs that are disseminating around the globe, warrants further development of this combination for future use against these pathogens.

Introduction

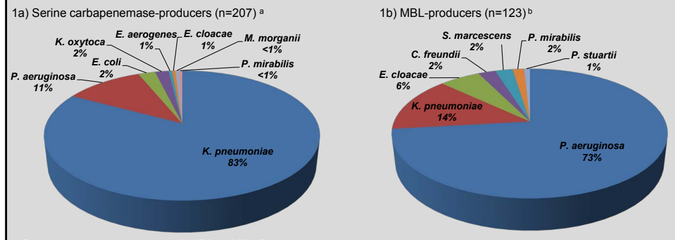
Avibactam (AVI) is a non-β-lactam β-lactamase inhibitor with activity against class A, class B, and some class D β-lactamases, including extended-spectrum β-lactamases (ESBLs) and KPCs. Aztreonam (ATM) is stable to hydrolysis by metallo-β-lactamases (MBL). ATM-AVI is being developed for use against carbapenem-resistant *Enterobacteriaceae*, especially those producing MBLs that often co-carry serine β-lactamases. This study evaluated the *in vitro* activity of ATM-AVI and comparators against *Enterobacteriaceae* and *P. aeruginosa* collected in 2014 in Europe.

Materials & Methods

- Non-duplicate clinical isolates were collected from 78 centers in 18 European countries.
- Organism identification and susceptibility testing were performed at a central laboratory using CLSI broth microdilution and interpreted using EUCAST 2015 breakpoints [1, 2]. Aztreonam-avibactam was tested at a fixed concentration of 4 mg/L avibactam.
- PCR and sequencing were used to determine the plasmid-mediated β-lactamase genes (ESBLs: SHV, TEM, CTX-M, VEB, PER, GES; original-spectrum β-lactamases: TEM-1, SHV-1, SHV-11; AmpC β-lactamases: ACC, ACT, CMY, DHA, FOX, MIR, MOX; carbapenemases: KPC, OXA-48-like, NDM, IMP, VIM, SPM, GIM) present in isolates that were non-susceptible to carbapenems (meropenem, imipenem, doripenem), phenotypically positive for ESBL, and those with ceftazidime MICs ≥ 16 mg/L [3].

Results

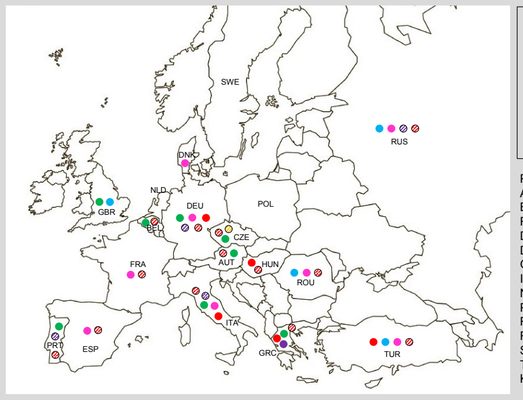
Figure 1a and 1b. Species distribution of Gram-negative isolates producing serine carbapenemases (KPC, OXA-48-like, GES) and metallo-β-lactamases (MBLs).



[a] Excludes three *K. pneumoniae* isolates carrying NDM-1 and OXA-48.

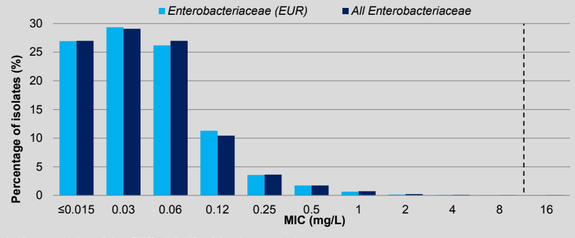
[b] β-lactamase content of 33 isolates of *Enterobacteriaceae* (n): ***C. freundii***, VIM-1 and SHV-12 (2); VIM-4, CTX-M-15 and TEM-OSBL (1). ***E. cloacae***, VIM-1 (1); VIM-1 and TEM-OSBL (5); NDM-1, CTX-M-15 and TEM-OSBL (1). ***K. pneumoniae***, VIM-1 and SHV-OSBL (8); VIM-4, CTX-M-15, SHV-OSBL and TEM-OSBL (2); NDM-1, CTX-M-15 and SHV-OSBL (2); NDM-1, OXA-48, CTX-M-15 and SHV-OSBL (3); NDM-1, CTX-M-15, CMY-6, SHV-OSBL, and TEM-OSBL (1); NDM-1, CTX-M-15, CMY-6, DHA-type, SHV-OSBL, and TEM-OSBL (1); NDM-16, CTX-M-15, SHV-12, and TEM-OSBL (1); NDM-16, CTX-M-15, SHV-OSBL, and TEM-OSBL (1). ***P. mirabilis***, VIM-1, VEB-1 and TEM-OSBL (1); VIM-1, CMY-16 and TEM-OSBL (1). ***P. stuartii***, VIM-1 and CMY-2 (1). ***S. marcescens***, VIM-5 (1); NDM-1, CTX-M-15 and TEM-OSBL (2).

Figure 2. Country distribution of carbapenemases detected in Gram-negative pathogens from Europe.



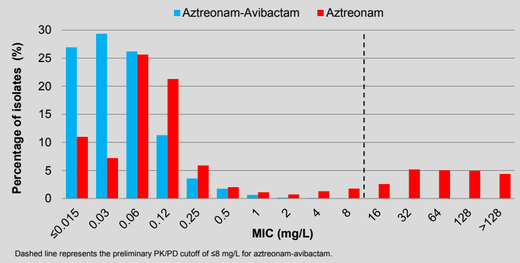
Participating countries: AUT, Austria; BEL, Belgium; CZE, Czech Republic; DNK, Denmark; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; NLD, Netherlands; POL, Poland; PRT, Portugal; ROU, Romania; RUS, Russia; ESP, Spain; SWE, Sweden; TUR, Turkey; GBR, United Kingdom.

Figure 3A. Aztreonam-avibactam MIC distributions against all *Enterobacteriaceae* collected globally (n=14,750) compared to isolates collected in Europe (n=7,453).



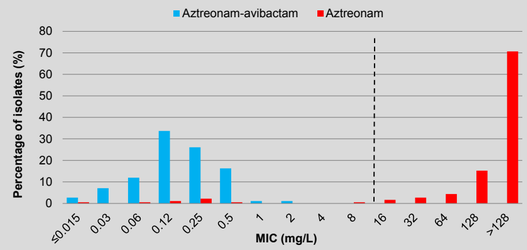
Dashed line represents the preliminary PK/PD cutoff of ≤8 mg/L for aztreonam-avibactam.
Not visible due to low numbers and scale of graph: isolates collected in Europe with aztreonam-avibactam MIC values of 2, 4, 8, and 16 mg/L (0.16%, 0.08%, 0.01%, and 0.03%, respectively); isolates collected globally with aztreonam-avibactam MIC values of 4, 8 and 16 mg/L (0.1%, 0.05% and 0.04%, respectively).

Figure 3B. Aztreonam and aztreonam-avibactam MIC distributions against *Enterobacteriaceae* (n=7,453) collected in Europe.



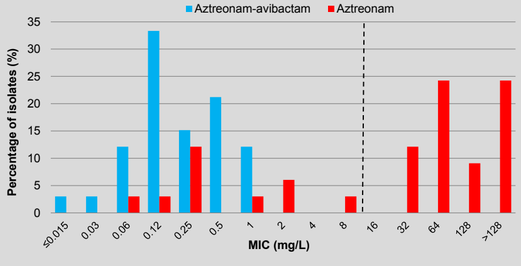
Dashed line represents the preliminary PK/PD cutoff of ≤8 mg/L for aztreonam-avibactam.

Figure 3C. Aztreonam and aztreonam-avibactam MIC distributions against serine carbapenemase-producing *Enterobacteriaceae* isolates (n=184) collected in Europe.



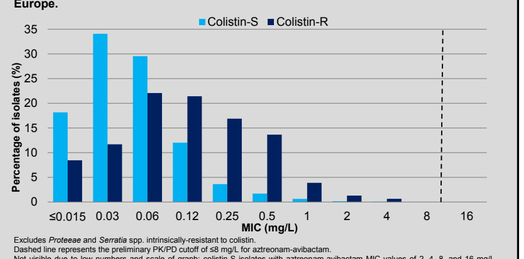
Dashed line represents the preliminary PK/PD cutoff of ≤8 mg/L for aztreonam-avibactam.
Includes isolates carrying KPC (n=112), OXA-48 (n=69), OXA-244 (n=1), GES (n=1), and KPC + GES (n=1).

Figure 3D. Aztreonam and aztreonam-avibactam MIC distributions against MBL-producing *Enterobacteriaceae* isolates (n=33) collected in Europe.



Dashed line represents the preliminary PK/PD cutoff of ≤8 mg/L for aztreonam-avibactam.
Includes isolates carrying VIM (n=21), NDM (n=9), and NDM + OXA-48 (n=3).

Figure 3E. Aztreonam-avibactam MIC distributions against colistin-susceptible (n=6,147) and colistin-resistant (n=154) *Enterobacteriaceae* isolates collected in Europe.



Excludes *Proteeae* and *Serratia* spp. intrinsically-resistant to colistin.
Dashed line represents the preliminary PK/PD cutoff of ≤8 mg/L for aztreonam-avibactam.
Not visible due to low numbers and scale of graph: colistin-S isolates with aztreonam-avibactam MIC values of 2, 4, 8, and 16 mg/L (0.15%, 0.07%, 0.02%, and 0.03%, respectively).

Table 1. *In vitro* activity of aztreonam-avibactam and comparator agents tested against *Enterobacteriaceae* and *P. aeruginosa* collected in Europe.

	MIC (mg/L)[a]			
Species/ phenotype/ genotype (n)	Range	MIC_{50}	MIC_{90}	%Susceptible[b]
Enterobacteriaceae All (7,453)				
Aztreonam-avibactam	≤0.015 - 16	0.03	0.12	NA[b]
Aztreonam	≤0.015 - >128	0.12	64	74.1
Cefepime	≤0.12 - >16	≤0.12	>16	77.4
Meropenem	≤0.004 - >8	0.03	0.12	97.3
Imipenem	≤0.03 - >8	0.25	2	91.9
Colistin	≤0.12 - >4	1	>4	82.7
Amikacin	≤0.25 - >32	2	8	93.8
Tigecycline	≤0.015 - 8	0.5	2	84.6
Levofloxacin	≤0.03 - >4	0.06	>4	75.1
ESBL-positive[c] (1,214)				
Aztreonam-avibactam	≤0.015 - 8	0.06	0.12	NA
Aztreonam	0.12 - >128	64	>128	1.6
Cefepime	≤0.12 - >16	>16	>16	3.2
Meropenem	0.015 - >8	0.06	0.12	98.6
Imipenem	0.06 - >8	0.25	0.5	97.5
Colistin	0.25 - >4	1	1	94.5
Amikacin	0.5 - >32	4	16	82.7
Tigecycline	0.03 - 8	0.5	2	85.2
Levofloxacin	≤0.03 - >4	>4	>4	30.1
Meropenem-NS[d] (203)				
Aztreonam-avibactam	≤0.015 - 4	0.25	0.5	NA
Aztreonam	0.06 - >128	>128	>128	4.4
Cefepime	0.25 - >16	>16	>16	1.5
Meropenem	4 - >8	>8	>8	0
Imipenem	0.5 - >8	>8	>8	9.9
Colistin	0.25 - >4	1	>4	72.4
Amikacin	1 - >32	32	>32	30.1
Tigecycline	0.12 - 8	1	2	70.9
Levofloxacin	≤0.03 - >4	>4	>4	7.4
KPC-positive[e] (113)				
Aztreonam-avibactam	≤0.015 – 2	0.12	0.5	NA
Aztreonam	8 - >128	>128	>128	0
Cefepime	0.5 - >16	>16	>16	0.9
Meropenem	0.5 - >8	>8	>8	0.9
Imipenem	1 - >8	>8	>8	0.9
Colistin	0.5 - >4	1	>4	71.7
Amikacin	1 - >32	32	>32	15.9
Tigecycline	0.12 - 8	1	4	67.3
Levofloxacin	≤0.03 - >4	>4	>4	5.3
OXA-48 positive[f] (69)				
Aztreonam-avibactam	≤0.015 – 1	0.12	0.5	NA
Aztreonam	≤0.015 - >128	>128	>128	13.0
Cefepime	≤0.12 - >16	>16	>16	10.1
Meropenem	0.25 - >8	4	>8	37.7
Imipenem	2 - >8	4	>8	21.7
Colistin	0.25 - >4	1	>4	72.5
Amikacin	1 - >32	4	>32	62.3
Tigecycline	0.12 - 8	1	2	73.9
Levofloxacin	0.06 - >4	>4	>4	13.0
VIM-positive[g] (21)				
Aztreonam-avibactam	≤0.015 – 1	0.12	0.5	NA
Aztreonam	0.06 - 128	32	64	33.3
Cefepime	0.5 - >16	>16	>16	4.8
Meropenem	0.25 - >8	8	>8	33.3
Imipenem	2 - >8	8	>8	4.8
Colistin	0.25 - >4	1	>4	81.0
Amikacin	1 - >32	8	16	71.4
Tigecycline	0.25 - 2	0.5	2	81.0
Levofloxacin	0.5 - >4	>4	>4	9.5

	MIC (mg/L)[a]			
Species/ phenotype/ genotype (n)	Range	MIC_{50}	MIC_{90}	%Susceptible[b]
NDM-positive[h] (12)				
Aztreonam-avibactam	0.12 – 1	0.25	1	NA[c]
Aztreonam	64 - 128	>128	>128	0.0
Cefepime	>16 - >16	>16	>16	0.0
Meropenem	>8 - >8	>8	>8	0.0
Imipenem	>8 - >8	>8	>8	0.0
Colistin	0.5 - >4	1	>4	75.0
Amikacin	8 - >32	>32	>32	8.3
Tigecycline	0.25 - 4	0.5	4	58.3
Levofloxacin	4 - >4	>4	>4	0.0
P. aeruginosa All (2,091)				
Aztreonam-avibactam	≤0.06 - >128	8	32	NA
Aztreonam	≤0.06 - >128	8	32	4.4
Cefepime	≤0.12 - >16	4	>16	76.9
Meropenem	≤0.06 - >8	1	>8	72.5
Imipenem	≤0.03 - >8	2	>8	69.7
Colistin	0.12 - >8	2	2	99.6
Amikacin	≤0.25 - >32	4	32	82.3
Levofloxacin	≤0.03 - >4	1	>4	61.8
GES-positive[i] (23)				
Aztreonam-avibactam	1 - 8	8	8	NA
Aztreonam	4 - 128	32	32	0.0
Cefepime	4 - >16	16	>16	30.4
Meropenem	>8 - >8	>8	>8	0.0
Imipenem	8 - >8	>8	>8	0.0
Colistin	0.5 - 4	2	2	100
Amikacin	16 - >32	32	>32	0.0
Levofloxacin	>4 - >4	>4	>4	0.0
IMP-positive[j] (5)				
Aztreonam-avibactam	32 – 64	-	-	0.0
Aztreonam	32 – 64	-	-	0.0
Cefepime	>16 - >16	-	-	0.0
Meropenem	>8 - >8	-	-	0.0
Imipenem	>8 - >8	-	-	0.0
Colistin	2 – 2	-	-	100
Amikacin	32 - 32	-	-	0.0
Levofloxacin	>4 - >4	-	-	0.0
VIM-positive[j] (85)				
Aztreonam-avibactam	4 - >128	16	32	NA
Aztreonam	4 - >128	16	32	0.0
Cefepime	8 - >16	>16	>16	1.2
Meropenem	4 - >8	>8	>8	0.0
Imipenem	8 - >8	>8	>8	0.0
Colistin	0.5 - 4	2	2	100
Amikacin	2 - >32	>32	>32	15.3
Levofloxacin	0.25 - >4	>4	>4	4.7

[a] MIC_{50} and MIC_{90} were not calculated for n <10.
[b]% Susceptibility was determined according to EUCAST interpretive criteria.
[c]NA, no breakpoints available.
[d]ESBL positive, isolates carrying extended-spectrum β-lactamases (ESBL) with or without original-spectrum β-lactamases (OSBL).
[e]NS, non-susceptible.
[f]Includes isolates carrying Class A (ESBLs, OSBLs) and/or Class C enzymes.
[g]Includes one isolate carrying KPC-2 and GES-6.
[h]Three isolates carrying OXA-48 and NDM-1 are included in the NDM-positive subset only.

Results Summary

- **330 carbapenemase-producing isolates (188 *K. pneumoniae*, 29 other isolates of *Enterobacteriaceae*, and 113 *P. aeruginosa*) of a total of 9544 tested were collected in 15 of 18 European countries in 2014, including isolates positive for KPC (n=112), VIM (n=106), OXA-48-like (n=70), GES (n=24), NDM (n=9), IMP (n=5), NDM and OXA-48 (n=3), and KPC and GES (n=1) enzymes.**
- **Though some carbapenemases appeared geographically restricted (e.g. NDM, IMP) others were widely disseminated, with KPC-positive and OXA-48-positive isolates each found in 8 countries and VIM-positive isolates found in 13 countries in Europe.**
- **The MIC distribution of aztreonam-avibactam (ATM-AVI) for isolates of *Enterobacteriaceae* collected in Europe was nearly identical to the distribution of MICs for isolates collected globally.**
- **ATM-AVI tested with MIC values ≤ 8 mg/L against 7451 (>99.9%) *Enterobacteriaceae* collected in Europe, including isolates carrying ESBLs, those carrying serine carbapenemases or MBLs with or without additional ESBL and AmpC β-lactamases, and colistin-resistant isolates.**
- **ATM-AVI showed only modest activity against *P. aeruginosa*.**

Conclusions

- **ATM-AVI had good activity against *Enterobacteriaceae* isolated in Europe, including difficult-to-treat carbapenemase-producing and colistin-resistant isolates.**
- **ATM-AVI tested with MIC values ≤1 mg/L against all MBL-containing *Enterobacteriaceae* and ≤2 mg/L against serine carbapenemase-containing *Enterobacteriaceae*, regardless of species or country of isolation.**
- **The promising *in vitro* activity of ATM-AVI against carbapenem-resistant *Enterobacteriaceae*, especially those producing MBLs that are disseminating around the globe, warrants further development of this combination for future use against these pathogens.**

References and Acknowledgments:

1. Clinical Laboratory Standards Institute. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standards -- Tenth Edition. CLSI document M07-910. Wayne, PA.
2. The European Committee on Antimicrobial Susceptibility Testing. 2015. Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0. http://www.eucast.org.
3. Lob SH, Kazmierczak KM, Badal RE, Hackel MA, Bouchillon SK, Biedenbach DJ, Sahm, DF. 2015. Trends in susceptibility of *Escherichia coli* from intra-abdominal Infections to ertapenem and comparators in the United States according to data from the SMART program, 2009 to 2013. Antimicrob Agents Chemother 59:3606-3610.

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