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In vitro activity of aztreonam-avibactam against isolates of *Enterobacteriaceae* collected in Europe as part of a global surveillance program, 2015

IHMA, Inc.
2122 Palmer Drive
Schaumburg, IL 60173 USA
Phone: +1.847.303.5003
Fax: +1.847.303.5601
www.ihmainc.com

K. M. Kazmierczak[1], H. Li[1], B. L. M. de Jonge[2], P. A. Bradford[2], D. F. Sahm[1] [1]International Health Management Associates, Schaumburg, IL, USA [2]Formerly of AstraZeneca Pharmaceuticals, Waltham, MA, USA

Abstract

Background: Aztreonam-avibactam is a β-lactam/β-lactamase inhibitor combination being developed for use against carbapenem-resistant *Enterobacteriaceae*, especially isolates producing metallo-β-lactamases (MBL). Aztreonam is stable to hydrolysis by MBL but inactivated by many serine β-lactamases. Avibactam is active against serine β-lactamases often co-carried with MBLs, including extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and serine carbapenemases. This study evaluated the *in vitro* activity of aztreonam-avibactam and comparators against *Enterobacteriaceae* collected in 2015 in Europe.
Materials/methods: Non-duplicate clinical isolates of *Enterobacteriaceae* were collected from 67 centres in 17 European countries. Susceptibility testing was performed using CLSI broth microdilution and interpreted using EUCAST breakpoints. Aztreonam-avibactam was tested at a fixed concentration of 4 mg/L avibactam. Multidrug resistant (MDR) was defined as resistant by EUCAST breakpoints to sentinel agents from three or more drug classes (cephalosporins, monobactams, β-lactam/β-lactamase inhibitor combinations, carbapenems, fluoroquinolones, aminoglycosides, glycylcyclines, and polymyxins). PCR and sequencing were used to determine the β-lactamase genes present in isolates with meropenem MIC >1 mg/L, ceftazidime MIC >8 mg/L, and those phenotypically positive for ESBL activity.
Results: Aztreonam-avibactam demonstrated good activity against *Enterobacteriaceae*, with an MIC_{90} of 0.12 mg/L against the overall population and MIC_{90}s of 0.5–1 mg/L against aztreonam non-susceptible, meropenem non-susceptible, colistin-resistant, MDR, and MBL-positive subsets (Table). >99.9% of isolates (6445 of 6449), including those that produced MBLs, were inhibited by ≤8 mg/L of aztreonam-avibactam. In comparison, 83.0–96.2% of overall isolates were susceptible to meropenem, tigecycline, and colistin. Activity of these comparator agents was greatly reduced against resistant subsets with the exception of colistin, which was active against MBL-positive isolates (MIC_{90} 1 mg/L, 96.7% susceptible). VIM- and NDM-type MBLs were found in 60 isolates of 10 species of *Enterobacteriaceae* collected in 10 European countries. 95.0% (n=57) of MBL-producing isolates co-carried one or more plasmid- or chromosomally-mediated ESBL, AmpC, or serine carbapenemase, including CTX-M-15, KPC-2 and OXA-48. No IMP-type MBLs were found in *Enterobacteriaceae* from Europe.

Species/Phenotype (n)	Drug (MIC_{90}/% Susceptible)									
	ATM-AVI		ATM		MER		CST		TGC	
Enterobacteriaceae , All (6449)	0.12	NA[a]	64	75.4%	0.12	96.2%	>8	83.0%	2	88.3%
ATM-NS (1590)	0.5	NA	>128	0.0%	>8	85.6%	4	89.8%	2	87.8%
MER-NS (248)	1	NA	>128	7.7%	>8	0.0%	>8	75.4%	2	76.2%
CST-R (125)[b]	1	NA	>128	24.0%	>8	51.2%	>8	0.0%	2	84.0%
MDR (1029)[c]	0.5	NA	>128	6.4%	>8	77.2%	>8	82.1%	4	80.0%
MBL-negative (6389)	0.12	NA	64	75.9%	0.12	97.0%	>8	82.9%	2	88.4%
MBL-positive (60)	1	NA	>128	21.7%	>8	10.0%	1	96.7%	2	70.0%

ATM-AVI, aztreonam-avibactam; ATM, aztreonam; MER, meropenem; CST, colistin; TGC, tigecycline; MDR, multidrug resistant; MBL, metallo-β-lactamase. EUCAST breakpoints were used to determine % susceptible and to define non-susceptible (NS) and resistant (R) subsets.
[a] NA, no breakpoints available
[b] Excludes isolates of Proteeae and *Serratia* spp.
[c] MDR, resistant by EUCAST breakpoints to sentinel agents from 3 or more drug classes.

Conclusions: Aztreonam-avibactam was highly potent *in vitro* against *Enterobacteriaceae* collected in Europe in 2015, including difficult-to-treat meropenem non-susceptible isolates (of which 21.8% carried MBLs), MDR, and colistin-resistant isolates. The promising activity of aztreonam-avibactam against MBL-producing isolates that often carry resistance mechanisms to multiple drug classes warrants further development of this combination for use against these pathogens.

Introduction

Aztreonam-avibactam is a β-lactam/β-lactamase inhibitor combination being developed for use against carbapenem-resistant *Enterobacteriaceae*, especially isolates producing metallo-β-lactamases (MBL). Aztreonam is stable to hydrolysis by MBL but inactivated by many serine β-lactamases. Avibactam is active against serine β-lactamases, including extended-spectrum β-lactamases (ESBL), AmpC β-lactamases, and serine carbapenemases, often co-carried in isolates with MBLs. This study evaluated the *in vitro* activity of aztreonam-avibactam and comparators against *Enterobacteriaceae* collected in 2015 in Europe.

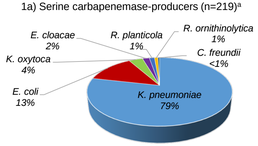
Materials & Methods

- 6,449 non-duplicate clinical isolates of *Enterobacteriaceae* were collected from 67 medical centres in 17 European countries in 2015.
- Susceptibility testing was performed using CLSI broth microdilution [1] and interpreted using EUCAST 2017 breakpoints [2]. Aztreonam-avibactam was tested at a fixed concentration of 4 mg/L avibactam.
- Multidrug resistant (MDR) was defined as resistant by EUCAST breakpoints to sentinel agents from three or more drug classes (cephalosporins, monobactams, β-lactam/β-lactamase inhibitor combinations, carbapenems, fluoroquinolones, aminoglycosides, glycylcyclines, and polymyxins).
- PCR and sequencing were used to determine the β-lactamase genes (*bla*) present in isolates with meropenem MIC >1 mg/L, ceftazidime MIC >8 mg/L, and those phenotypically positive for ESBL activity.
- Isolates were screened for *bla* encoding MBLs (bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{GIM}), serine carbapenemases (bla_{KPC}, $bla_{OXA-48-like}$, bla_{GES}), ESBLs (bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{VEB}, bla_{PER}, bla_{GES}), and AmpC enzymes (bla_{ACC}, bla_{ACT}, bla_{CMY}, bla_{DHA}, bla_{FOX}, bla_{MIR}, bla_{MOX}) as previously described [3].

Results

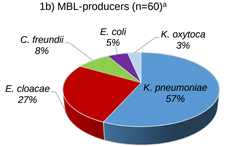
Figure 1a-1d. Species distribution of serine carbapenemase-positive (KPC, OXA-48-like, GES), metallo-β-lactamase-positive, colistin-resistant, and multidrug resistant (MDR) *Enterobacteriaceae* isolates.

1a) Serine carbapenemase-producers (n=219)[a]



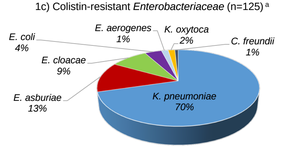
[a] Excludes two *K. pneumoniae* isolates carrying VIM-26 and KPC-2, five *C. freundii* carrying VIM-31 and OXA-48, and one *E. cloacae* and eleven *K. pneumoniae* carrying NDM-1 and OXA-48.

1b) MBL-producers (n=60)[a]



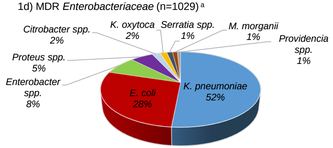
[a] β-lactamase content of 60 isolates of *Enterobacteriaceae* (n): ***C. freundii***, VIM-31, OXA-48 and TEM-OSBL (5). ***E. cloacae,*** VIM-1 and TEM-OSBL (7); VIM-1 (3), NDM-1 and CTX-M-15 (3); NDM-1 (1); NDM-1 and OXA-48 (1); NDM-1, CTX-M-15, SHV-12 and TEM-OSBL (1). ***E. coli,*** NDM-1 and TEM-OSBL (1); NDM-1, CTX-M-27 and CMY-2 (1); NDM-1, CTX-M-15, CTX-M-27, CMY-6, DHA-1 and TEM-OSBL (1). ***K. oxytoca,*** VIM-1 (1); VIM-1, CTX-M-3 and SHV-12 (1). ***K. pneumoniae,*** NDM-1, CTX-M-15 and SHV-OSBL (7); VIM-26 and SHV-5 (6); NDM-1, OXA-48, CTX-M-15 and SHV-ESBL (5); NDM-1, OXA-48 and CTX-M-15 (3); NDM-1, OXA-48 and SHV-OSBL (3); NDM-1, CTX-M-15, SHV-OSBL and TEM-OSBL (3); VIM-26, KPC-2, SHV-12 and TEM-OSBL (2); VIM-1 and SHV-OSBL (2); VIM-4, CTX-M-15, SHV-OSBL and TEM-OSBL (1); NDM-1, CTX-M-15, SHV-5, CMY-6, and TEM-OSBL (1); NDM-1, CMY-6 and SHV-OSBL (1).

1c) Colistin-resistant *Enterobacteriaceae* (n=125)[a]



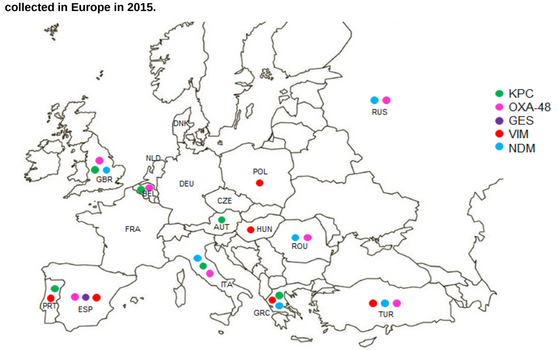
[a] Excludes Proteeae and *Serratia* spp. intrinsically resistant to colistin.

1d) MDR *Enterobacteriaceae* (n=1029)[a]



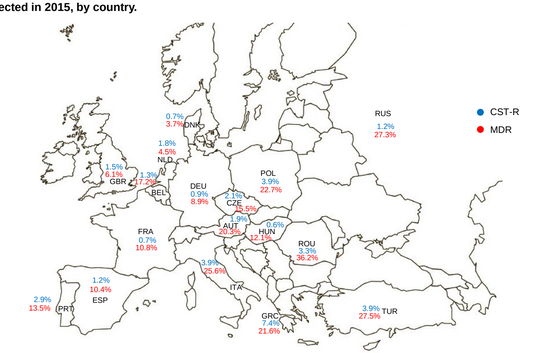
[a]*Citrobacter* spp. includes *Citrobacter freundii* (18), *Citrobacter braakii* (1), and *Citrobacter koseri* (1). *Enterobacter* spp. includes *Enterobacter cloacae* (61), *Enterobacter aerogenes* (15), *Enterobacter asburiae* (3), and *Enterobacter kobei* (2). *Proteus* spp. includes *Proteus mirabilis* (52) and *Proteus vulgaris* (4). *Providencia* spp. includes *Providencia stuartii* (7) and *Providencia rettgeri* (1). *Serratia* spp. includes *Serratia marcescens* (13) and *Serratia liquefaciens* (1).

Figure 2A. Country distribution of carbapenemases detected in *Enterobacteriaceae* clinical isolates collected in Europe in 2015.



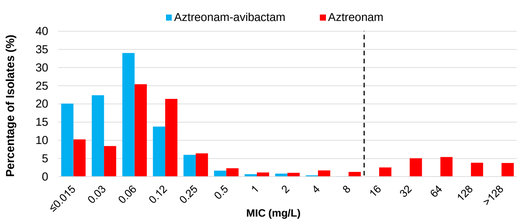
Participating countries: AUT, Austria; BEL, Belgium; CZE, Czech Republic; DNK, Denmark; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; NLD, Netherlands; POL, Poland; PRT, Portugal; ROU, Romania; RUS, Russia; ESP, Spain; TUR, Turkey; GBR, United Kingdom.

Figure 2B. Percentages of colistin-resistant (CST-R) and multidrug resistant (MDR) *Enterobacteriaceae* collected in 2015, by country.



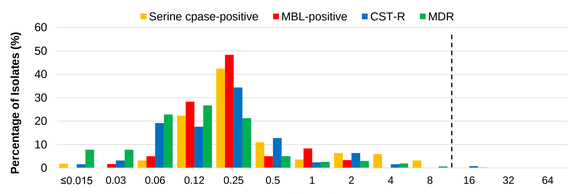
Participating countries: AUT, Austria; BEL, Belgium; CZE, Czech Republic; DNK, Denmark; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; NLD, Netherlands; POL, Poland; PRT, Portugal; ROU, Romania; RUS, Russia; ESP, Spain; TUR, Turkey; GBR, United Kingdom.

Figure 3A. Aztreonam and aztreonam-avibactam MIC distributions against *Enterobacteriaceae* (n=6,449) collected in Europe.



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 with aztreonam-avibactam MIC values of 8 (0.11%), 16 (0.05%), and 64 mg/L (0.02%).

Figure 3B. Aztreonam-avibactam MIC distributions against serine carbapenemase-positive (Serine cpase-positive) (n=219), MBL-positive (n=60), colistin-resistant (CST-R) (n=125), and multidrug resistant (MDR) (n=1029) *Enterobacteriaceae* isolates collected in Europe.



Dashed line represents the preliminary PK/PD cutoff of ≤8 mg/L for aztreonam-avibactam. CST-R *Enterobacteriaceae* exclude isolates of Proteeae and *Serratia* spp. intrinsically resistant to colistin. One CST-R *K. pneumoniae* isolate carrying SHV-12 and TEM-OSBL β-lactamases tested with an aztreonam-avibactam MIC of 16 mg/L. Not visible due to low numbers and scale of graph: MDR isolates with aztreonam-avibactam MIC values of 16 (0.19%) and 64 mg/L (0.10%).

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

Group (n)[a] /Drug	MIC (mg/L)			% Susceptible[b]
	Range	MIC_{50}	MIC_{90}	
Enterobacteriaceae All (6,449)				
Aztreonam-avibactam	≤0.015-64	0.06	0.12	NA[c]
Aztreonam	≤0.015->128	0.12	64	75.4
Cefepime	≤0.12->16	≤0.12	>16	79.1
Meropenem	≤0.004->8	0.06	0.12	**96.2**
Colistin	≤0.06->8	0.5	>8	83.0
Amikacin	≤0.25->32	2	8	**93.4**
Tigecycline	≤0.015-8	0.5	2	88.3
Levofloxacin	≤0.004->8	0.06	>8	72.2
Aztreonam-NS (1,590)				
Aztreonam-avibactam	≤0.015-64	0.12	0.5	NA
Aztreonam	2->128	64	>128	0.0
Cefepime	≤0.12->16	>16	>16	19.9
Meropenem	0.008->8	0.06	>8	85.6
Colistin	0.12->8	0.5	4	89.8
Amikacin	≤0.25->32	4	32	79.5
Tigecycline	≤0.015-8	0.5	2	87.8
Levofloxacin	0.008->8	8	>8	30.4
Meropenem-NS (248)				
Aztreonam-avibactam	≤0.015-16	0.25	1	NA
Aztreonam	0.06->128	>128	>128	7.7
Cefepime	≤0.12->16	>16	>16	3.2
Meropenem	4->8	>8	>8	0.0
Colistin	0.25->8	1	>8	75.4
Amikacin	0.5->32	32	>32	33.5
Tigecycline	0.12-8	1	2	76.2
Levofloxacin	0.06->8	>8	>8	6.5
Colistin-R (125)				
Aztreonam-avibactam	≤0.015-16	0.25	1	NA
Aztreonam	0.03->128	128	>128	24.0
Cefepime	≤0.12->16	>16	>16	30.4
Meropenem	0.015->8	1	>8	51.2
Colistin	4->8	>8	>8	0.0
Amikacin	0.5->32	8	>32	51.2
Tigecycline	0.12-8	1	2	84.0
Levofloxacin	0.015->8	>8	>8	30.4
MDR (1,029)				
Aztreonam-avibactam	≤0.015-64	0.12	0.5	NA
Aztreonam	≤0.015->128	64	>128	6.4
Cefepime	≤0.12->16	>16	>16	6.8
Meropenem	0.008->8	0.06	>8	77.2
Colistin	0.12->8	0.5	>8	82.1
Amikacin	0.5->32	8	>32	68.5
Tigecycline	≤0.015-8	0.5	4	80.0
Levofloxacin	0.03->8	>8	>8	8.2
MBL-negative (6,389)				
Aztreonam-avibactam	≤0.015-64	0.06	0.12	NA
Aztreonam	≤0.015->128	0.12	64	75.9
Cefepime	≤0.12->16	≤0.12	>16	79.8
Meropenem	≤0.004->8	0.06	0.12	**97.0**
Colistin	≤0.06->8	0.5	>8	82.9
Amikacin	≤0.25->32	2	8	**94.0**
Tigecycline	≤0.015-8	0.5	2	88.4
Levofloxacin	≤0.004->8	0.06	>8	72.8
MBL-positive (60)[d]				
Aztreonam-avibactam	0.03-2	0.25	1	NA
Aztreonam	0.06->128	128	>128	21.7
Cefepime	1->16	>16	>16	3.3
Meropenem	0.25->8	>8	>8	10.0
Colistin	0.25->8	0.5	1	**96.7**
Amikacin	1->32	32	>32	28.3
Tigecycline	0.12-4	0.5	2	70.0
Levofloxacin	0.03->8	>8	>8	10.0

[a] NS, non-susceptible; R, resistant; MDR, multidrug resistant; MBL, metallo-β-lactamase.
[b] % Susceptibility was determined according to EUCAST 2017 interpretive criteria. Values ≥90% are indicated in bold.
[c] NA, no breakpoints available.
[d] Includes isolates carrying Class A (KPC, ESBL, OSBL) and/or Class C and/or Class D (OXA-48-like) enzymes.

Results Summary

- 279 carbapenemase-producing isolates (206 *K. pneumoniae*, 32 *E. coli*, 20 *E. cloacae*, and 21 other isolates of *Enterobacteriaceae*) of a total of 6,449 tested were collected in 12 of 17 participating European countries in 2015, including isolates positive for KPC (n=111), OXA-48-like (n=107), VIM (n=21), NDM (n=20), GES (n=1), NDM and OXA-48-like (n=12), VIM and OXA-48-like (n=5), and VIM and KPC (n=2) enzymes (Figure 1 and Figure 2).
- Though no *Enterobacteriaceae* carrying IMP were found, other carbapenemases were widely disseminated, with OXA-48-positive isolates found in 7 countries and KPC-positive, VIM-positive, and NDM-positive isolates each found in 6 countries. Multiple carbapenemase types were found in 9 European countries (Figure 2).
- Aztreonam-avibactam showed good activity against all *Enterobacteriaceae* (MIC_{90}, 0.12 mg/L) collected in Europe. >99.9% (6,445 of 6,449) of isolates tested with aztreonam-avibactam MIC values ≤8 mg/L, including isolates carrying MBLs and/or serine β-lactamases, aztreonam-NS, meropenem-NS, colistin-R, and MDR isolates (MIC_{90}s, 0.5–1 mg/L) (Figure 3 and Table 1).
- Among meropenem-NS, colistin-R, MDR, and MBL-positive isolates, susceptibilities to other tested agents were reduced (cephalosporins, 3.2–30.4% susceptible; meropenem, 0–77.2% susceptible; amikacin, 28.3–68.5% susceptible; tigecycline, 70.0–84.0% susceptible). Colistin was active against MBL-positive isolates (MIC_{90}, 1 mg/L; 96.7% susceptible) but showed decreased activity against other difficult-to-treat subsets of isolates (0–82.1% susceptible) (Table 1).

Conclusions

- Aztreonam-avibactam was highly potent *in vitro* against *Enterobacteriaceae* collected in Europe in 2015, including difficult-to-treat meropenem non-susceptible isolates (of which 21.8% carried MBLs), MDR, and colistin-resistant isolates.
- The promising activity of aztreonam-avibactam against MBL-producing isolates that often carry resistance mechanisms to multiple drug classes warrants further development of this combination for 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.
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This study was sponsored by AstraZeneca (AZ). The AZ product aztreonam-avibactam was acquired by Pfizer in December 2016. IHMA received financial support from AZ in connection with the study and development of this poster. K.M. Kazmierczak, H. Li and D. Sahm are employees of IHMA. B.L.M de Jonge, an employee of and shareholder in AZ at the time of the study, is currently an employee of Pfizer. P.A Bradford was an employee of and shareholder in AZ at the time of this study.