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In vitro activity of aztreonam-avibactam against metallo-β-lactamase-producing *Enterobacteriaceae* isolates collected during a global surveillance program, 2012–2015

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], M. Hackel[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 in development for use against infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE), especially isolates carrying metallo-β-lactamases (MBLs). Aztreonam is refractory to hydrolysis by MBLs but is inactivated by Class A (KPC, extended-spectrum β-lactamases) and plasmid-mediated or stably-derepressed chromosomally-encoded Class C serine β-lactamases. Avibactam inhibits the activities of Class A, C, and some Class D β-lactamases that are frequently co-carried with MBLs. MBL-positive isolates often carry mechanisms conferring resistance against other antimicrobial classes such as aminoglycosides and fluoroquinolones, and are increasingly found among species naturally resistant to colistin and tigecycline, further reducing available therapeutic options. This study evaluated the *in vitro* activity of aztreonam-avibactam and comparators against MBL-positive isolates of *Enterobacteriaceae* collected globally in 2012–2015. **Materials/methods:** 51,352 non-duplicate isolates of *Enterobacteriaceae* were collected from 208 medical centres in 40 countries. Susceptibility testing was performed by CLSI broth microdilution. Aztreonam-avibactam was tested at a fixed concentration of 4 mg/L avibactam. PCR and sequencing of β-lactamase genes was performed on isolates with meropenem, doripenem, or imipenem MIC >1 mg/L or ertapenem MIC >0.5 mg/L. **Results:** 3343 isolates of *Enterobacteriaceae* were molecularly characterized. Genes encoding IMP, VIM or NDM were detected in 267 isolates (149 *Klebsiella* spp., 65 *Enterobacter* spp., 18 *Citrobacter freundii*, 18 Proteeae, 12 *Escherichia coli*, and 5 *Serratia marcescens*). 45% of MBL-positive isolates were collected in 3 countries (Greece, n=55; the Philippines, n=35; Romania, n=31), with the remaining 55% collected in 25 countries each contributing 1–15 MBL-positive isolates. 86.9% of isolates co-carried MBLs and one or more Class A or Class C β-lactamase able to hydrolyze aztreonam, resulting in MIC_{90} values of ≥128 mg/L for this agent (Table). In contrast, aztreonam-avibactam demonstrated potent *in vitro* activity against these MBL-positive isolates of *Enterobacteriaceae*, with MIC_{90} values of 0.5–1 mg/L against NDM-, IMP- and VIM-positive isolates (Table). All 267 MBL-positive isolates were inhibited by ≤8 mg/L of aztreonam-avibactam.

Organism (n)[a]	Drug	Cumulative percentage of isolates inhibited at each MIC (mg/L) (%):														
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
MBL-positive, All (267)	ATM	4.9	6.0	9.0	19.5	23.2	26.2	28.5	29.2	30.3	33.7	42.3	59.6	84.3	**100**
	ATM-AVI	16.9	27.7	54.7	82.0	89.5	**95.9**	98.1	99.3	100					
IMP-positive (29)	ATM		6.9	6.9	20.7	24.1	27.6	27.6	31.0	34.5	34.5	51.7	65.5	89.7	**100**
	ATM-AVI	20.7	34.5	51.7	82.8	89.7	**93.1**	93.1	100						
VIM-positive (96)	ATM	7.3	9.4	15.6	29.2	34.4	39.6	42.7	42.7	44.8	49.0	60.4	79.2	**90.6**	100
	ATM-AVI	25.0	29.2	49.0	76.0	85.4	**96.9**	100							
NDM-positive (142)	ATM	4.2	3.5	4.9	12.7	15.5	16.9	19.0	19.7	19.7	23.2	28.2	45.1	78.9	**100**
	ATM-AVI	10.6	25.4	59.2	85.9	**92.3**	95.8	97.9	98.6	100					

MBL-positive, gene encoding a metallo-β-lactamase (MBL) was detected by PCR; ATM, aztreonam; ATM-AVI, aztreonam-avibactam; MIC_{90} is indicated in bold font.
[a] Includes isolates co-carrying class A, C, and D β-lactamases.

Conclusions: Aztreonam-avibactam was highly active *in vitro* against all genotypically identified MBL-containing *Enterobacteriaceae*, regardless of serine β-lactamase co-carriage, species or country of isolation. The emergence and increasingly widespread dissemination of MBLs among *Enterobacteriaceae*, including ESKAPE pathogens and species that are intrinsically resistant to last-in-line therapies such as colistin and/or tigecycline, warrants further development of aztreonam-avibactam to explore therapy against infections caused by CRE.

Introduction

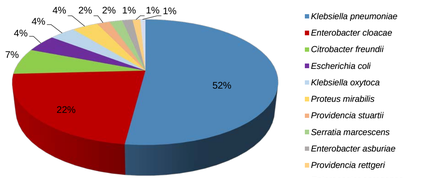
Aztreonam-avibactam is in development for use against infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE), especially isolates carrying metallo-β-lactamases (MBLs). Aztreonam is refractory to hydrolysis by MBLs but is inactivated by Class A (KPC, extended-spectrum β-lactamases) and plasmid-mediated or stably-derepressed chromosomally-encoded Class C serine β-lactamases. Avibactam inhibits the activities of Class A, C, and some Class D β-lactamases that are frequently co-carried with MBLs. MBL-positive isolates often carry mechanisms conferring resistance against other antimicrobial classes such as aminoglycosides and fluoroquinolones, and are increasingly found among species naturally resistant to colistin and tigecycline, further reducing available therapeutic options against these isolates. This study evaluated the *in vitro* activity of aztreonam-avibactam and comparators against MBL-positive isolates of *Enterobacteriaceae* collected globally in 2012–2015.

Materials & Methods

- 51,352 non-duplicate isolates of *Enterobacteriaceae* from intra-abdominal, urinary tract, respiratory tract, skin and soft tissue, and bloodstream (2014-2015 only) infections were collected from 208 medical centres in 40 countries.
- Susceptibility testing was performed by Clinical Laboratory and Standards Institute (CLSI) broth microdilution [1] and interpreted using EUCAST 2017 guidelines [2]. Aztreonam-avibactam was tested at a fixed concentration of 4 mg/L avibactam.
- PCR and sequencing of β-lactamase genes (*bla*$_{NDM}$, *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{SPM}$, bla$_{GIM}$, *bla*$_{KPC}$, *bla*$_{OXA-48-like}$, *bla*$_{GES}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{VEB}$, *bla*$_{PER}$, *bla*$_{ACC}$, *bla*$_{ACT}$, *bla*$_{CMY}$, *bla*$_{DHA}$, *bla*$_{FOX}$, *bla*$_{MIR}$, *bla*$_{MOX}$) was performed on 3,343 isolates with meropenem, doripenem, or imipenem MICs >1 mg/L or ertapenem MICs >0.5 mg/L as described previously [3].

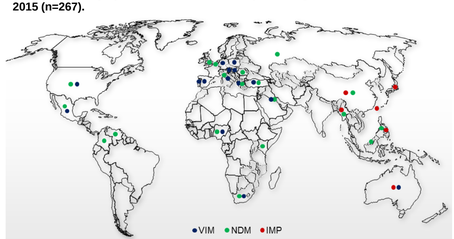
Results

Figure 1. Species distribution of *Enterobacteriaceae* isolates producing MBLs (n=267)[a].



[a]Includes isolates carrying NDM-1 and OXA-48 (n=15), NDM-1 and OXA-232 (n=7), VIM-31 and OXA-48 (n=6),VIM-4 and OXA-48 (n=2), VIM-1 and KPC-2 (n=4), VIM-26 and KPC-2 (n=2), and IMP-4 and KPC-2 (n=2).

Figure 2A. Geographic distribution of MBL-producing isolates collected in 2012-2015 (n=267).

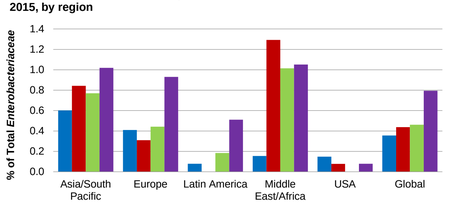


Countries in which MBL-positive *Enterobacteriaceae* isolates were detected (no. of isolates): Australia (3), Austria (2), Belgium (1), China (10), Colombia (4), Germany (1), Greece (55), Hungary (5), Italy (6), Japan (2), Kenya (4), Kuwait (13), Malaysia (2), Mexico (11), Nigeria (15), Philippines (35), Poland (1), Portugal (1), Romania (31), Russia (9), South Africa (9), Spain (5), Taiwan (7), Thailand (15), Turkey (12), United Kingdom (2), United States (4), and Venezuela (2).

Participating countries in which MBL-positive *Enterobacteriaceae* isolates were not detected: Argentina, Brazil, Chile, Czech Republic, Denmark, France, Hong Kong, Ireland, Israel, Netherlands, South Korea, Sweden.

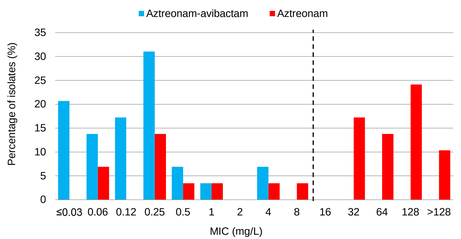
No isolates were obtained from patients in China in 2014-2015.

Figure 2B. Proportion of MBL-positive *Enterobacteriaceae* collected in 2012-2015, by region



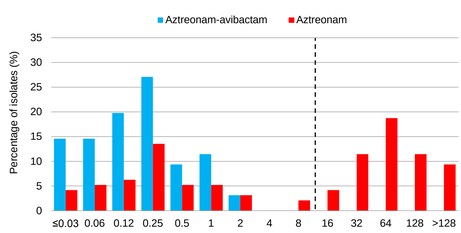
The proportion of MBL-positive *Enterobacteriaceae* isolates identified in each region and year should not be taken as an estimate of prevalence because this global surveillance study was not designed to be a prevalence study.

Figure 3A. Aztreonam and aztreonam-avibactam MIC distributions against *Enterobacteriaceae* isolates producing IMP-type MBLs (n=29).



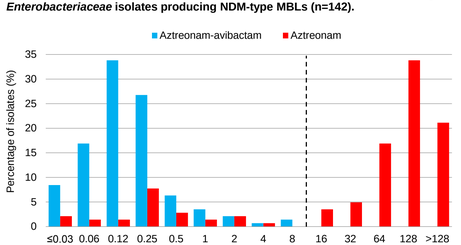
Dashed line represents the preliminary PK/PD cutoff of ≤8 mg/L for aztreonam-avibactam.

Figure 3B. Aztreonam and aztreonam-avibactam MIC distributions against *Enterobacteriaceae* isolates producing VIM-type MBLs (n=96).



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

Figure 3C. Aztreonam and aztreonam-avibactam MIC distributions against *Enterobacteriaceae* isolates producing NDM-type MBLs (n=142).



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

Table 1. *In vitro* activity of aztreonam-avibactam and comparator agents tested against *Enterobacteriaceae* isolates producing MBLs.

Group (n)[a] / Drug	MIC (mg/L)[b]			
	Range	MIC_{50}	MIC_{90}	% Susceptible[c]
All MBL-positive *Enterobacteriaceae* (267)				
Aztreonam-avibactam	≤0.015 to 8	0.12	1	NA[d]
Aztreonam	≤0.015 to >128	64	>128	26.2
Colistin (172)[e]	0.25 to >4	1	>4	87.8
Tigecycline	0.06 to >8	1	4	65.5
MBL only (6)[f]				
Aztreonam-avibactam	≤0.015 to 0.06	--	--	NA
Aztreonam	≤0.015 to 1	--	--	**100**
Colistin (0)[e]	--	--	--	--
Tigecycline	1 to 2	--	--	16.7
MBL + OSBL (24)				
Aztreonam-avibactam	0.03 to 0.5	0.12	0.25	NA
Aztreonam	0.06 to 64	0.25	2	87.5
Colistin (14)[e]	0.5 to >4	1	>4	78.6
Tigecycline	0.25 to 2	0.5	2	79.2
MBL + ESBL ± OSBL (95)				
Aztreonam-avibactam	≤0.015 to 0.5	0.12	0.25	NA
Aztreonam	0.06 to >128	128	>128	4.2
Colistin (67)[e]	0.25 to >4	1	2	**92.5**
Tigecycline	0.06 to 8	1	2	68.4
MBL + AmpC ± OSBL (61)				
Aztreonam-avibactam	≤0.015 to 8	0.25	2	NA
Aztreonam	≤0.015 to >128	1	64	50.8
Colistin (38)[e]	0.25 to >4	1	>4	78.9
Tigecycline	0.25 to 8	1	2	70.5
MBL + ESBL + AmpC ± OSBL (43)				
Aztreonam-avibactam	≤0.015 to 2	0.12	0.5	NA
Aztreonam	0.5 to >128	128	>128	4.6
Colistin (25)[e]	0.25 to >4	0.5	>4	48.8
Tigecycline	0.12 to >8	1	4	51.2
MBL + KPC (1)				
Aztreonam-avibactam	0.5	--	--	NA
Aztreonam	>128	--	--	0.0
Colistin (0)[e]	--	--	--	--
Tigecycline	1	--	--	**100**
MBL + KPC + ESBL ± OSBL (4)				
Aztreonam-avibactam	0.5 to 2	--	--	NA
Aztreonam	>128 to >128	--	--	0.0
Colistin (2)[e]	1 to 1	--	--	**100**
Tigecycline	1 to 2	--	--	50.0
MBL + KPC + AmpC + OSBL (1)				
Aztreonam-avibactam	0.5	--	--	NA
Aztreonam	>128	--	--	0.0
Colistin (0)[e]	--	--	--	--
Tigecycline	0.5	--	--	**100**
MBL + KPC + ESBL + AmpC + OSBL (2)				
Aztreonam-avibactam	0.5 to 0.5	--	--	NA
Aztreonam	>128 to >128	--	--	0.0
Colistin (0)[e]	--	--	--	--
Tigecycline	2 to 2	--	--	0.0
MBL + OXA-48-like + OSBL (5)[g]				
Aztreonam-avibactam	0.12 to 2	--	--	NA
Aztreonam	0.25 to 1	--	--	**100**
Colistin (4)[e]	0.5 to 1	--	--	**100**
Tigecycline	0.5 to 2	--	--	60.0
MBL + OXA-48-like + ESBL ± OSBL (16)[g]				
Aztreonam-avibactam	0.12 to 1	0.25	0.5	NA
Aztreonam	128 to >128	>128	>128	0.0
Colistin (16)[e]	0.25 to >4	0.5	2	**93.7**
Tigecycline	0.5 to 4	1	2	62.5
MBL + OXA-48-like + AmpC ± OSBL (8)				
Aztreonam-avibactam	0.25 to 1	--	--	NA
Aztreonam	0.25 to 32	--	--	12.5
Colistin (6)[e]	0.25 to 1	--	--	**100**
Tigecycline	0.25 to 4	--	--	87.5
MBL + OXA-48-like + ESBL + AmpC + OSBL (1)				
Aztreonam-avibactam	0.25	--	--	NA
Aztreonam	128	--	--	0.0
Colistin (0)[e]	--	--	--	--
Tigecycline	1	--	--	**100**

[a]OSBL, original-spectrum β-lactamase (includes TEM-1, SHV-1, and SHV-11); ESBL, extended-spectrum β-lactamase (includes SHV, CTX-M-type, VEB, and the endogenous ESBL common to *K. oxytoca*); AmpC, class C cephalosporin (includes plasmid-mediated AmpCs and chromosomally-encoded AmpCs common to *C. freundii, Enterobacter* spp., *Providencia* spp., and *S. marcescens*.)
[b] --, MIC_{50} and MIC_{90} were not calculated for n <10 isolates.
[c]Susceptibility percentages were determined using EUCAST 2017 breakpoints. Values ≥90% are indicated in bold.
[d]NA, not applicable (no breakpoint defined).
[e]Values are for isolates collected in 2014-2015 only.
[f]Does not include species with endogenous AmpC or ESBL enzymes.
[g]Includes isolates carrying OXA-48 or OXA-232.

Results Summary

- Of the 51,352 *Enterobacteriaceae* collected as part of a global surveillance study from 2012–2015, 267 isolates of 11 species produced IMP-, VIM- and NDM-type MBLs and were collected in 28 of 40 participating countries located in all regions (Europe, EUR; Asia/South Pacific, AP; Middle East/Africa, MEA; Latin America, LA; North America, NA) (Figure 1 and Figure 2A).
- 74% of MBL-positive isolates were collected in 9 countries: Greece (n=55), the Philippines (n=35), Romania (n=31), Thailand (n=15), Nigeria (n=15), Kuwait (n=13), Turkey (n=12), Mexico (n=11), and China (n=10). The remaining 26% were collected from 19 countries (1–9 isolates each) (Figure 2A).
- IMP-positive *Enterobacteriaceae* isolates were only found in AP. NDM-positive isolates were found in multiple countries in EUR, MEA, AP, LA, and the United States. VIM-positive isolates were found in multiple countries in MEA and EUR and in one country each from AP (Australia), LA (Mexico), and NA (United States) (Figure 2A).
- While the incidence of MBL-producing isolates is still relatively low, increasing numbers of MBL-producing isolates were collected globally from 2012–2015 (Figure 2B).
- Aztreonam-avibactam demonstrated potent *in vitro* activity against MBL-positive *Enterobacteriaceae* isolates, with MIC_{90} values of 1 mg/L, 1 mg/L, and 0.5 mg/L against IMP-, VIM-, and NDM-positive isolates, respectively (Abstract table and Figure 3).
- All MBL-positive isolates tested with aztreonam-avibactam MIC values ≤8 mg/L, including isolates carrying ESBLs, AmpC β-lactamases, and serine carbapenemases in all combinations (Table 1).
- Aztreonam-avibactam was more active, based on MIC_{90} values, than tigecycline and colistin against the overall collection of MBL-positive isolates and against subsets of isolates carrying MBLs with different combinations of serine β-lactamases (Table 1).

Conclusions

- Aztreonam-avibactam was highly active *in vitro* against all genotypically identified MBL-containing *Enterobacteriaceae*, regardless of serine β-lactamase co-carriage, species or country of isolation.
- The emergence and increasingly widespread dissemination of MBLs among *Enterobacteriaceae*, including ESKAPE pathogens and species that are intrinsically resistant to last-in-line therapies such as colistin and/or tigecycline, warrants further development of aztreonam-avibactam to explore therapy against infections caused by CRE.

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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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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