In Vitro Activity of Aztreonam-Avibactam against *Enterobacteriaceae* and *Pseudomonas aeruginosa* Isolated by Clinical Laboratories in 40 Countries from 2012 to 2015

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ABSTRACT The combination of the monobactam aztreonam and the non- β -lactam β -lactamase inhibitor avibactam is currently in clinical development for the treatment of serious infections caused by metallo- β -lactamase (MBL)-producing *Enterobacteriaceae*, a difficult-to-treat subtype of carbapenem-resistant *Enterobacteriaceae* for which therapeutic options are currently very limited. The present study tested clinically significant isolates of *Enterobacteriaceae* ($n = 51,352$) and *Pseudomonas aeruginosa* ($n = 11,842$) collected from hospitalized patients in 208 medical center laboratories from 40 countries from 2012 to 2015 for *in vitro* susceptibility to aztreonam-avibactam, aztreonam, and comparator antimicrobial agents using a standard broth microdilution methodology. Avibactam was tested at a fixed concentration of 4 $\mu\text{g/ml}$ in combination with 2-fold dilutions of aztreonam. The MIC₉₀s of aztreonam-avibactam and aztreonam were 0.12 and 64 $\mu\text{g/ml}$, respectively, for all *Enterobacteriaceae* isolates; >99.9% of all isolates and 99.8% of meropenem-nonsusceptible isolates ($n = 1,498$) were inhibited by aztreonam-avibactam at a concentration of $\leq 8 \mu\text{g/ml}$. PCR and DNA sequencing identified 267 *Enterobacteriaceae* isolates positive for MBL genes (NDM, VIM, IMP); all *Enterobacteriaceae* carrying MBLs demonstrated aztreonam-avibactam MICs of $\leq 8 \mu\text{g/ml}$ and a MIC₉₀ of 1 $\mu\text{g/ml}$. Against all *P. aeruginosa* isolates tested, the MIC₉₀ of both aztreonam-avibactam and aztreonam was 32 $\mu\text{g/ml}$; against MBL-positive *P. aeruginosa* isolates ($n = 452$), MIC₉₀ values for aztreonam-avibactam and aztreonam were 32 and 64 $\mu\text{g/ml}$, respectively. The current study demonstrated that aztreonam-avibactam possesses potent *in vitro* activity against a recent, sizeable global collection of *Enterobacteriaceae* clinical isolates, including isolates that were meropenem nonsusceptible, and against MBL-positive isolates of *Enterobacteriaceae*, for which there are few treatment options.

KEYWORDS aztreonam-avibactam, Gram-negative bacteria, metallo- β -lactamase, surveillance studies

Carbapenem resistance in *Enterobacteriaceae* has been reported to arise from one or a combination of the following mechanisms: carbapenemase production; reduced outer membrane permeability, often in the milieu of an extended-spectrum β -lactamase (ESBL), AmpC, or multiple β -lactamases; efflux across the outer membrane; or mutations in penicillin binding proteins (PBP) (1–5). Of these mechanisms, carbapenemase production is regarded as the most worrisome because many of the genes encoding carbapenemases reside on plasmids, creating the potential for hori-

Received 4 March 2017 Returned for modification 10 April 2017 Accepted 9 June 2017

Accepted manuscript posted online 19 June 2017

Citation Karlowsky JA, Kazmierczak KM, de Jonge BLM, Hackel MA, Sahm DF, Bradford PA. 2017. *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 61:e00472-17. <https://doi.org/10.1128/AAC.00472-17>.

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zontal spread. Carbapenemases are a diverse collection of β -lactamases and include members of Ambler class A β -lactamases (e.g., *Klebsiella pneumoniae* carbapenemase [KPC], GES), class B metallo- β -lactamases (MBLs; e.g., NDM, VIM, IMP, SPM), and class D β -lactamases (e.g., OXA) (6). MBLs in particular are problematic because they hydrolyze all classes of β -lactams except monobactams (aztreonam) and are not inhibited by classic serine β -lactamase inhibitors (clavulanic acid, tazobactam, sulbactam).

Aztreonam, a monobactam, is a unique agent among currently marketed β -lactams, in that it is stable to hydrolysis by MBLs. Unfortunately, aztreonam is easily inactivated by Ambler class A β -lactamases, including ESBLs and KPCs, and plasmid-encoded or stably derepressed chromosomally encoded class C (AmpC) β -lactamases (6–8). Gram-negative bacilli (GNB) carrying an MBL also commonly carry additional β -lactamases, including ESBLs, AmpC, or serine carbapenemases (i.e., KPCs), that inactivate aztreonam, negating the activity of aztreonam against these isolates (9). Over time, the susceptibility of *Enterobacteriaceae* to aztreonam has been reduced (6, 7).

Avibactam is a bridged diazabicyclooctanone, non- β -lactam β -lactamase inhibitor of a wide range of serine β -lactamases that offers a broader β -lactamase inhibition profile than current β -lactamase inhibitors (clavulanic acid, tazobactam, and sulbactam), which inactivate only specific class A enzymes (3, 10). Avibactam protects β -lactams from hydrolysis by class A enzymes (including TEM, CTX-M ESBLs, and KPCs), class C enzymes (e.g., CMY, ACT, FOX), and some class D enzymes (e.g., OXA-48, OXA-139) (2, 10–13). When combined with avibactam, aztreonam is able to inhibit cell wall synthesis in MBL-producing bacteria, despite the presence of cocarried serine β -lactamases (2, 9, 14).

To better define the inhibitory profile of aztreonam-avibactam, a recent (2012 to 2015) global collection of clinically significant isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* from hospitalized patients was generated. The current study determined the profiles of *in vitro* susceptibility to aztreonam-avibactam, aztreonam, and comparator antimicrobial agents of these isolates using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (15, 16). The study also identified carbapenem-nonsusceptible and MBL-positive isolates of *Enterobacteriaceae* in the collection and focused attention on the activity of aztreonam-avibactam against isolates with these important emerging resistant phenotypes and genotypes. This study is an expansion of one previously published that examined isolates from 2012 and 2013 only (9).

RESULTS

Table 1 displays the *in vitro* activities of aztreonam-avibactam, aztreonam, and the comparator antimicrobial agents tested against all *Enterobacteriaceae* isolates collected in the five geographic regions from 2012 to 2015. For all regions, aztreonam-avibactam was the most potent agent tested, with a MIC₉₀ of 0.12 μ g/ml for all *Enterobacteriaceae* isolates in four (Asia/South Pacific, Europe, Middle East/Africa, and North America) of the five geographic regions (Table 1). The MIC₉₀ of aztreonam-avibactam for *Enterobacteriaceae* isolates collected in Latin America was 1 dilution higher at 0.25 μ g/ml. In comparison, the MIC₉₀ of (and percent susceptibility to) aztreonam ranged from 16 μ g/ml (87.6% susceptible) for isolates from North America to 128 μ g/ml (68.0% susceptible) for isolates from Latin America. Only meropenem showed activity comparable to that of aztreonam-avibactam against these isolates (MIC₉₀ = 0.12 μ g/ml). Other comparator agents showed much higher MIC₉₀ values (≥ 2 μ g/ml), resulting from the presence of a subpopulation of isolates with intrinsic resistance (*Proteeae* for tigecycline and colistin) or acquired resistance (the remainder of the compounds).

Table 1 also summarizes regional data for MBL-negative and MBL-positive *Enterobacteriaceae* isolates from each region. MBL-positive isolates constituted 1.0% (41/4,232) of isolates from the Middle East/Africa region, 0.8% (74/9,149) of isolates from Asia/South Pacific, 0.5% (131/24,826) of isolates from Europe, 0.2% (17/7,665) of isolates from Latin America, and 0.1% (4/5,480) of isolates from North America. Against MBL-positive isolates of *Enterobacteriaceae*, the MIC₉₀ of aztreonam-avibactam ranged from

TABLE 1 Regional activities of aztreonam-avibactam, aztreonam, and comparator antimicrobial agents tested against 51,352 clinical isolates of *Enterobacteriaceae* categorized by MBL status

Region and category (n ^a)	Drug	MIC (μ g/ml)			% of isolates with MIC interpretation of susceptible ^a
		50%	90%	Range	
Global (51,352)	Aztreonam-avibactam	0.06	0.12	≤ 0.015 to >128	NA ^b
	Aztreonam	0.12	64	≤ 0.015 to >128	76.0
	Meropenem	0.03	0.12	≤ 0.004 to >8	97.1
	Cefepime	≤ 0.12	>16	≤ 0.12 to >16	79.0
	Ceftazidime	0.25	64	≤ 0.015 to >128	77.0
	Piperacillin-tazobactam	2	64	≤ 0.25 to >128	84.6
	Amikacin	2	8	≤ 0.25 to >32	96.7
	Tigecycline	0.5	2	≤ 0.015 to >8	93.5
	Levofloxacin	0.06	>4	≤ 0.03 to >4	76.1
	Colistin (n = 27,836) ^c	1	>4	≤ 0.12 to >4	83.2
MBL negative ^d (51,085)	Aztreonam-avibactam	0.06	0.12	≤ 0.015 to >128	NA
	Aztreonam	0.12	64	≤ 0.015 to >128	76.3
	Meropenem	0.03	0.12	≤ 0.004 to >8	97.6
	Cefepime	≤ 0.12	>16	≤ 0.12 to >16	79.4
	Ceftazidime	0.25	64	≤ 0.015 to >128	77.4
	Piperacillin-tazobactam	2	64	≤ 0.25 to >128	85.0
	Amikacin	2	8	≤ 0.25 to >32	96.9
	Tigecycline	0.5	2	≤ 0.015 to >8	93.6
	Levofloxacin	0.06	>4	≤ 0.03 to >4	76.3
	Colistin (n = 27,664)	1	>4	≤ 0.12 to >4	83.2
MBL positive ^e (267)	Aztreonam-avibactam	0.12	1	≤ 0.015 to 8	NA
	Aztreonam	64	>128	≤ 0.015 to >128	29.2
	Meropenem	>8	>8	0.25 to >8	6.4
	Cefepime	>16	>16	≤ 0.12 to >16	4.9
	Ceftazidime	>128	>128	0.25 to >128	1.5
	Piperacillin-tazobactam	>128	>128	0.5 to >128	6.0
	Amikacin	16	>32	0.5 to >32	57.7
	Tigecycline	1	4	0.06 to 8	89.1
	Levofloxacin	>4	>4	≤ 0.03 to >4	28.8
	Colistin (n = 172)	1	>4	0.25 to >4	87.8
Asia/South Pacific (9,149)	Aztreonam-avibactam	0.06	0.12	≤ 0.015 to 32	NA
	Aztreonam	0.12	64	≤ 0.015 to >128	73.9
	Meropenem	0.03	0.12	≤ 0.004 to >8	98.4
	Cefepime	≤ 0.12	>16	≤ 0.12 to >16	77.8
	Ceftazidime	0.25	64	≤ 0.015 to >128	75.1
	Piperacillin-tazobactam	2	64	≤ 0.25 to >128	86.9
	Amikacin	2	8	≤ 0.25 to >32	97.5
	Tigecycline	0.5	2	≤ 0.015 to >8	93.9
	Levofloxacin	0.12	>4	≤ 0.03 to >4	74.6
	Colistin (n = 4,140)	0.5	>4	≤ 0.12 to >4	83.0
MBL negative (9,075)	Aztreonam-avibactam	0.06	0.12	≤ 0.015 to 32	NA
	Aztreonam	0.12	64	≤ 0.015 to >128	74.3
	Meropenem	0.03	0.12	≤ 0.004 to >8	99.1
	Cefepime	≤ 0.12	>16	≤ 0.12 to >16	78.4
	Ceftazidime	0.25	64	≤ 0.015 to >128	75.7
	Piperacillin-tazobactam	2	32	≤ 0.25 to >128	87.5
	Amikacin	2	8	≤ 0.25 to >32	97.8
	Tigecycline	0.5	2	≤ 0.015 to >8	94.0
	Levofloxacin	0.12	>4	≤ 0.03 to >4	74.9
	Colistin (n = 4,103)	0.5	>4	≤ 0.12 to >4	82.9
MBL positive (74)	Aztreonam-avibactam	0.12	0.5	≤ 0.015 to 8	NA
	Aztreonam	64	>128	≤ 0.015 to >128	25.7
	Meropenem	>8	>8	0.5 to >8	8.1
	Cefepime	>16	>16	1 to >16	2.7
	Ceftazidime	>128	>128	32 to >128	0
	Piperacillin-tazobactam	>128	>128	0.5 to >128	16.2
	Amikacin	4	>32	0.5 to >32	66.2
	Tigecycline	1	4	0.06 to 8	89.2
	Levofloxacin	>4	>4	0.06 to >4	36.5
	Colistin (n = 37)	1	>4	0.25 to >4	86.5
Europe (24,826)	Aztreonam-avibactam	0.06	0.12	≤ 0.015 to >128	NA
	Aztreonam	0.12	64	≤ 0.015 to >128	77.4

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TABLE 1 (Continued)

Region and category (<i>n</i> ^a)	Drug	MIC (μ g/ml)			% of isolates with MIC interpretation of susceptible ^a
		50%	90%	Range	
MBL negative (24,695)	Meropenem	0.03	0.12	≤ 0.004 to >8	96.8
	Cefepime	≤ 0.12	>16	≤ 0.12 to >16	80.4
	Ceftazidime	0.25	64	≤ 0.015 to >128	78.1
	Piperacillin-tazobactam	2	128	≤ 0.25 to >128	83.5
	Amikacin	2	8	≤ 0.25 to >32	96.3
	Tigecycline	0.5	2	≤ 0.015 to >8	93.1
	Levofloxacin	0.06	>4	≤ 0.03 to >4	78.3
	Colistin (<i>n</i> = 13,902)	1	>4	≤ 0.12 to >4	82.8
	Aztreonam-avibactam	0.06	0.12	≤ 0.015 to >128	NA
	Aztreonam	0.12	64	≤ 0.015 to >128	77.7
	Meropenem	0.03	0.12	≤ 0.004 to >8	97.3
	Cefepime	≤ 0.12	>16	≤ 0.12 to >16	80.8
	Ceftazidime	0.25	64	≤ 0.015 to >128	78.5
	Piperacillin-tazobactam	2	128	≤ 0.25 to >128	83.9
	Amikacin	2	8	≤ 0.25 to >32	96.6
MBL positive (131)	Tigecycline	0.5	2	≤ 0.015 to >8	93.1
	Levofloxacin	0.06	>4	≤ 0.03 to >4	78.7
	Colistin (<i>n</i> = 13,809)	1	>4	≤ 0.12 to >4	82.8
	Aztreonam-avibactam	0.25	1	≤ 0.015 to 2	NA
	Aztreonam	64	>128	0.06 to >128	29.8
	Meropenem	>8	>8	0.25 to >8	7.6
	Cefepime	>16	>16	≤ 0.12 to >16	5.3
	Ceftazidime	>128	>128	16 to >128	0
	Piperacillin-tazobactam	>128	>128	16 to >128	2.3
	Amikacin	16	>32	1 to >32	51.1
	Tigecycline	1	4	0.12 to >8	88.6
	Levofloxacin	>4	>4	≤ 0.03 to >4	13.7
	Colistin (<i>n</i> = 93)	0.5	2	0.25 to >4	90.3
Latin America (7,665)	Aztreonam-avibactam	0.06	0.25	≤ 0.015 to 16	NA
	Aztreonam	0.12	128	≤ 0.015 to >128	68.0
	Meropenem	0.03	0.12	≤ 0.004 to >8	94.9
	Cefepime	≤ 0.12	>16	≤ 0.12 to >16	70.4
	Ceftazidime	0.25	64	≤ 0.015 to >128	69.9
	Piperacillin-tazobactam	4	>128	≤ 0.25 to >128	81.1
	Amikacin	2	8	≤ 0.25 to >32	94.9
	Tigecycline	0.5	2	≤ 0.015 to >8	93.7
	Levofloxacin	0.25	>4	≤ 0.03 to >4	67.5
	Colistin (<i>n</i> = 4,516)	0.5	>4	≤ 0.12 to >4	83.0
MBL negative (7,648)	Aztreonam-avibactam	0.06	0.25	≤ 0.015 to 16	NA
	Aztreonam	0.12	128	≤ 0.015 to >128	68.0
	Meropenem	0.03	0.12	≤ 0.004 to >8	95.1
	Cefepime	≤ 0.12	>16	≤ 0.12 to >16	70.5
	Ceftazidime	0.25	64	≤ 0.015 to >128	70.0
	Piperacillin-tazobactam	4	128	≤ 0.25 to >128	81.3
	Amikacin	2	8	≤ 0.25 to >32	95.0
	Tigecycline	0.5	2	≤ 0.015 to >8	93.8
	Levofloxacin	0.25	>4	≤ 0.03 to >4	67.6
	Colistin (<i>n</i> = 4,500)	0.5	>4	≤ 0.12 to >4	83.1
MBL positive (17)	Aztreonam-avibactam	0.12	0.25	≤ 0.015 to 1	NA
	Aztreonam	16	128	0.03 to >128	47.1
	Meropenem	8	>8	1 to >8	5.9
	Cefepime	>16	>16	1 to >16	5.9
	Ceftazidime	>128	>128	0.25 to >128	5.9
	Piperacillin-tazobactam	>128	>128	1 to >128	5.9
	Amikacin	16	>32	2 to >32	64.7
	Tigecycline	1	4	0.5 to 4	82.4
	Levofloxacin	1	>4	0.25 to >4	58.8
	Colistin (<i>n</i> = 16)	1	>4	0.25 to >4	68.8
Middle East/Africa (4,232)	Aztreonam-avibactam	0.03	0.12	≤ 0.015 to >128	NA
	Aztreonam	0.12	64	≤ 0.015 to >128	72.3
	Meropenem	0.03	0.12	≤ 0.004 to >8	98.0
	Cefepime	≤ 0.12	>16	≤ 0.12 to >16	72.6

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TABLE 1 (Continued)

Region and category (<i>n</i> ^a)	Drug	MIC (μ g/ml)			% of isolates with MIC interpretation of susceptible ^a
		50%	90%	Range	
MBL negative (4,191)	Ceftazidime	0.25	64	≤ 0.015 to >128	73.4
	Piperacillin-tazobactam	4	64	≤ 0.25 to >128	85.1
	Amikacin	2	8	≤ 0.25 to >32	97.1
	Tigecycline	0.5	2	≤ 0.015 to >8	94.5
	Levofloxacin	0.12	>4	≤ 0.03 to >4	74.8
	Colistin (<i>n</i> = 2,429)	1	>4	≤ 0.12 to >4	83.7
	Aztreonam-avibactam	0.03	0.12	≤ 0.015 to >128	NA
	Aztreonam	0.12	64	≤ 0.015 to >128	72.8
	Meropenem	0.03	0.12	≤ 0.004 to >8	99.0
	Cefepime	≤ 0.12	>16	≤ 0.12 to >16	73.3
	Ceftazidime	0.25	64	≤ 0.015 to >128	74.0
	Piperacillin-tazobactam	2	64	≤ 0.25 to >128	86.0
	Amikacin	2	8	≤ 0.25 to >32	97.4
	Tigecycline	0.5	2	≤ 0.015 to >8	94.5
	Levofloxacin	0.12	>4	≤ 0.03 to >4	75.0
	Colistin (<i>n</i> = 2,404)	1	>4	≤ 0.12 to >4	83.6
MBL positive (41)	Aztreonam-avibactam	0.12	0.25	≤ 0.015 to 8	NA
	Aztreonam	64	128	≤ 0.015 to >128	24.4
	Meropenem	>8	>8	2 to >8	0
	Cefepime	>16	>16	≤ 0.12 to >16	7.3
	Ceftazidime	>128	>128	0.5 to >128	7.3
	Piperacillin-tazobactam	>128	>128	32 to >128	0
	Amikacin	8	>32	1 to >32	63.4
	Tigecycline	1	2	0.25 to 8	92.7
	Levofloxacin	2	>4	0.06 to >4	51.2
	Colistin (<i>n</i> = 25)	1	2	0.25 to >4	92.0
North America (5,480)	Aztreonam-avibactam	0.03	0.12	≤ 0.015 to 16	NA
	Aztreonam	0.12	16	≤ 0.015 to >128	87.6
	Meropenem	0.03	0.12	≤ 0.004 to >8	98.5
	Cefepime	≤ 0.12	1	≤ 0.12 to >16	91.5
	Ceftazidime	0.25	16	≤ 0.015 to >128	87.6
	Piperacillin-tazobactam	2	16	≤ 0.25 to >128	90.6
	Amikacin	2	4	≤ 0.25 to >32	98.8
	Tigecycline	0.5	2	≤ 0.015 to >8	93.7
	Levofloxacin	0.06	>4	≤ 0.03 to >4	81.5
	Colistin (<i>n</i> = 2,849)	1	>4	≤ 0.12 to >4	85.2
	Aztreonam-avibactam	0.03	0.12	≤ 0.015 to 16	NA
	Aztreonam	0.12	16	≤ 0.015 to >128	87.6
	Meropenem	0.03	0.12	≤ 0.004 to >8	98.6
	Cefepime	≤ 0.12	1	≤ 0.12 to >16	91.6
	Ceftazidime	0.25	16	≤ 0.015 to >128	87.7
	Piperacillin-tazobactam	2	16	≤ 0.25 to >128	90.7
MBL negative (5,476)	Amikacin	2	4	≤ 0.25 to >32	98.9
	Tigecycline	0.5	2	≤ 0.015 to >8	93.7
	Levofloxacin	0.06	>4	≤ 0.03 to >4	81.6
	Colistin (<i>n</i> = 2,848)	1	>4	≤ 0.12 to >4	85.2
	Aztreonam-avibactam	ND ^f	ND	0.06 to 0.5	NA
	Aztreonam	ND	ND	0.12 to >128	50.0
	Meropenem	ND	ND	8 to >8	0
	Cefepime	ND	ND	>16	0
	Ceftazidime	ND	ND	>128	0
	Piperacillin-tazobactam	ND	ND	>128	0
	Amikacin	ND	ND	16 to >32	25.0
	Tigecycline	ND	ND	0.5 to 2	100
	Levofloxacin	ND	ND	0.5 to >4	25.0
	Colistin (<i>n</i> = 1)	ND	ND	1	100

^aMICs were interpreted according to CLSI breakpoints (16), with the exception of those for aztreonam-avibactam, for which clinical breakpoints have not yet been assigned; tigecycline, for which MIC interpretative criteria published by the U.S. FDA were used (33); and colistin, for which EUCAST breakpoints were applied (34).

^bNA, not available (no breakpoint criteria has been defined for aztreonam-avibactam).

^cColistin was tested without added surfactant; data are for isolates collected in 2014 and 2015 only.

^dMBL negative, no gene encoding a metallo- β -lactamase included in the screening algorithm was detected by PCR. This isolate type includes carbapenem-sensitive and carbapenem-nonsusceptible metallo- β -lactamase-negative isolates.

^eMBL positive, a gene encoding a metallo- β -lactamase was detected by PCR. This isolate type can also carry one or more additional serine β -lactamases.

^fND, not determined; i.e., the MIC₅₀s and MIC₉₀s were not calculated when there were <10 isolates.

^g*n*, number of isolates tested, unless indicated otherwise.

TABLE 2 MIC cumulative frequency distribution for aztreonam-avibactam and aztreonam tested against clinical isolates of *Enterobacteriaceae* and *P. aeruginosa* collected worldwide from 2012 to 2015

Organism (<i>n</i> ^d) and drug	% frequency distribution by MIC (μ g/ml) ^a :														
	≤ 0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
<i>Enterobacteriaceae</i>															
All (51,352)															
Aztreonam-avibactam	20.9	48.7	79.1	91.4	96.3	98.3	99.2	99.7	99.9	>99.9	>99.9	>99.9	>99.9	>99.9	100
Aztreonam	9.1	16.0	41.2	62.6	69.8	72.1	73.4	74.4	76.0	77.8	80.7	86.0	92.0	96.1	100
Meropenem nonsusceptible (1,498)															
Aztreonam-avibactam	2.5	5.9	15.2	39.0	72.4	88.2	94.0	97.5	99.2	99.8	99.9	100			
Aztreonam	0.5	0.6	1.3	2.5	5.3	6.5	7.1	7.7	8.5	9.1	10.9	14.8	24.5	42.1	100
Meropenem nonsusceptible, MBL negative ^b (1,248)															
Aztreonam-avibactam	2.2	5.0	13.1	36.1	70.6	87.9	93.6	97.4	99.2	99.8	99.9	100			
Aztreonam	0.2	0.3	0.6	1.4	2.6	3.2	3.4	3.8	4.5	5.0	6.4	9.5	17.9	33.8	100
Meropenem nonsusceptible, MBL positive ^c (250)															
Aztreonam-avibactam	4.0	10.4	25.6	53.6	81.6	89.6	96.0	98.4	99.2	100					
Aztreonam	2.0	2.0	5.2	8.0	18.8	22.8	25.6	27.6	28.4	29.6	33.2	41.2	57.2	83.2	100
All MBL-positive isolates (267)															
Aztreonam-avibactam	4.9	12.0	27.7	54.7	82.0	89.5	95.9	98.1	99.3	100					
Aztreonam	2.2	2.6	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
<i>P. aeruginosa</i>															
All (11,842)															
Aztreonam-avibactam			0.6	0.7	2.2	3.7	4.7	8.7	42.0	73.4	86.9	96.3	99.2	99.7	100
Aztreonam			0.2	0.3	1.3	2.7	3.6	6.7	34.4	61.7	77.4	90.6	95.8	97.7	100
MBL positive (452)															
Aztreonam-avibactam					0.2	0.4	0.4	1.1	13.9	38.1	70.6	90.7	97.3	98.7	100
Aztreonam					0.2	0.4	0.4	0.9	10.4	24.8	59.7	83.8	90.5	92.9	100

^aFor each MIC distribution, MIC₉₀ values are in bold.^bMBL negative, no gene encoding a metallo- β -lactamase included in the screening algorithm was detected by PCR. This isolate type includes carbapenem-sensitive and carbapenem-nonsusceptible metallo- β -lactamase-negative isolates.^cMBL positive, a gene encoding a metallo- β -lactamase was detected by PCR. This isolate type can also carry one or more additional serine β -lactamases.^d*n*, number of isolates tested.

0.25 μ g/ml (Latin America and Middle East/Africa) to 1 μ g/ml (Europe). The MIC of aztreonam-avibactam was ≤ 8 μ g/ml for all MBL-positive *Enterobacteriaceae* (Table 2).

Aztreonam-avibactam and meropenem were the two most potent antimicrobial agents tested (i.e., they had the lowest MIC₉₀ values) against MBL-negative isolates of *Enterobacteriaceae*, with their MIC₉₀s being equal to or within 1 2-fold dilution. However, against MBL-positive *Enterobacteriaceae*, aztreonam-avibactam was >8- to >32-fold more potent (by comparison of MIC₉₀ values) than meropenem against isolates from Asia/South Pacific, Europe, Latin America, and the Middle East/Africa (Table 1). The percentages of isolates susceptible to antimicrobial agents other than aztreonam-avibactam were markedly lower for MBL-positive *Enterobacteriaceae* isolates than for MBL-negative isolates for agents other than tigecycline and colistin (Table 1).

Avibactam lowered the MIC₉₀ of aztreonam for all *Enterobacteriaceae* isolates tested by 512-fold, from 64 μ g/ml to 0.12 μ g/ml (Table 2). A concentration of aztreonam-avibactam of ≤ 8 μ g/ml inhibited >99.9% (51,327/51,352) of all *Enterobacteriaceae* isolates (Table 2). Aztreonam-avibactam remained very potent against meropenem-nonsusceptible *Enterobacteriaceae* isolates (MIC₉₀ of 1 μ g/ml), regardless of the presence or absence of an MBL. Twenty-five *Enterobacteriaceae* isolates tested with aztreonam-avibactam MICs ranging from 16 to >128 μ g/ml, and all were MBL negative. These included isolates of *Escherichia coli* (*n* = 8; 5 of these isolates carried CMY-type AmpC β -lactamases), *Enterobacter cloacae* (*n* = 5), *Klebsiella pneumoniae* (*n* = 4), *Proteus mirabilis* (*n* = 2), and one isolate each of *Enterobacter aerogenes*, *Morganella morganii*, *Proteus vulgaris*, *Providencia rettgeri*, *Raoultella ornithinolytica*, and *Serratia marcescens*, collected in 21 hospitals in 14 countries (Argentina, Belgium, China, Denmark, Germany, Italy, Kenya, Kuwait, Portugal, Russia, Spain, Taiwan, Thailand, Venezu-

ela). The mechanism(s) responsible for the increased MICs of aztreonam-avibactam in these 25 isolates remains undefined; however, 22 and 16 of the 25 isolates were susceptible to meropenem and ceftazidime-avibactam, respectively (data not shown).

Table 3 provides a summary of the *in vitro* activities of aztreonam-avibactam, aztreonam, and the other antimicrobial agents tested against *Enterobacteriaceae* isolates collected in 2012 and 2013 compared with the activities against isolates collected in 2014 and 2015. The MIC₉₀ of aztreonam-avibactam was consistent across both the 2012 and 2013 and the 2014 and 2015 data sets, with a MIC₉₀ of 0.12 μ g/ml for MBL-negative isolates of *Enterobacteriaceae* and with a MIC₉₀ of 0.5 to 1 μ g/ml for MBL-positive isolates.

Table 4 describes the comparative MICs of aztreonam-avibactam and aztreonam alone against the 267 isolates of MBL-positive *Enterobacteriaceae* (with or without additional serine β -lactamase enzymes) identified in this study. The 267 isolates with an MBL (139 *K. pneumoniae*, 59 *E. cloacae*, 18 *Citrobacter freundii*, 12 *E. coli*, 10 *Klebsiella oxytoca*, 10 *P. mirabilis*, 5 *Providencia stuartii*, 5 *S. marcescens*, 4 *Enterobacter asburiae*, 3 *P. rettgeri*, and 2 *E. aerogenes* isolates) were composed of 142 isolates with NDM, 96 isolates with VIM, and 29 isolates with IMP. The majority of the characterized isolates with an MBL also produced ESBLs, class C enzymes, and/or serine carbapenemases. For isolates carrying only an MBL ($n = 6$), aztreonam alone inhibited all isolates at a concentration of 1 μ g/ml. However, for isolates with an MBL plus one or more serine β -lactamases, the majority of isolates produced aztreonam MICs of >128 μ g/ml. Aztreonam-avibactam was much more potent against these isolates, with all isolates being inhibited by a concentration of 8 μ g/ml and with MIC₉₀s, where calculable, ranging from 0.5 to 2 μ g/ml, emphasizing the benefit of combining avibactam with aztreonam against isolates harboring serine β -lactamases in addition to an MBL. The 237 isolates that coproduced an MBL and an ESBL, AmpC, and/or serine carbapenemase produced aztreonam-avibactam and aztreonam MIC₉₀ values of 1 μ g/ml (MIC range, ≤ 0.015 to 8 μ g/ml) and >128 μ g/ml (MIC range, ≤ 0.015 to >128 μ g/ml), respectively.

Aztreonam alone consistently demonstrated low potency against most *Enterobacteriaceae* isolates that produced class A or class C enzymes alone or in combination with an MBL. Of particular note were isolates that expressed a serine carbapenemase in combination with an MBL. There were eight isolates that produced both an MBL and a KPC enzyme (six *K. pneumoniae* isolates cocarrying KPC-2 and VIM-1 [$n = 4$] or KPC-2 and VIM-26 [$n = 2$] were collected in two hospitals in Greece, and two *K. oxytoca* isolates cocarrying KPC-2 and IMP-4 were collected in one hospital in China). Thirty additional isolates produced an MBL and an OXA-48-like enzyme: 21 *K. pneumoniae* isolates cocarried NDM-1 and OXA-48 ($n = 14$, two hospitals in Romania in 2014 and 2015) or NDM-1 and OXA-232 (one hospital each in Thailand [$n = 5$; 2015], Belgium [$n = 1$; 2013], and Kuwait [$n = 1$; 2015]), 5 isolates of *C. freundii* cocarried VIM-31 and OXA-48 (Turkey, one hospital in 2015), and 4 *E. cloacae* isolates cocarried VIM-4 and OXA-48 ($n = 2$, 1 hospital in Kuwait), VIM-31 and OXA-48 ($n = 1$, Turkey), or NDM-1 and OXA-48 ($n = 1$, Romania). All of these isolates were inhibited by aztreonam-avibactam at a concentration of 0.12 to 2 μ g/ml.

Against 11,842 clinical isolates of *P. aeruginosa* collected worldwide from 2012 to 2015, the MIC₉₀s for both aztreonam-avibactam and aztreonam alone were 32 μ g/ml (Table 2). Against MBL-positive *P. aeruginosa* isolates, the MIC₉₀ of aztreonam-avibactam (32 μ g/ml) was 1 2-fold dilution lower than that of aztreonam alone (64 μ g/ml); the MIC distributions for aztreonam-avibactam and aztreonam alone were similar for all *P. aeruginosa* isolates and MBL-positive isolates. In comparison, the MIC₉₀ values of meropenem and colistin were >8 μ g/ml and 2 μ g/ml, respectively, against all and MBL-positive *P. aeruginosa* (data not shown).

DISCUSSION

The monobactam- β -lactamase inhibitor combination aztreonam-avibactam is in development to treat serious infections caused by MBL-producing *Enterobacteriaceae*, a difficult-to-treat subtype of carbapenem-resistant *Enterobacteriaceae* for which ther-

TABLE 3 Longitudinal analysis of *in vitro* activities of aztreonam-avibactam, aztreonam, and comparative antimicrobial agents tested against clinical isolates of *Enterobacteriaceae* collected worldwide in 2012 and 2013 and in 2014 and 2015 and categorized by MBL status

Time period and category (<i>n</i> ^f)	Drug	MIC (μg/ml)			% of isolates with MIC interpretation of susceptible ^a
		50%	90%	Range	
2012–2013					
All (23,516)	Aztreonam-avibactam	0.06	0.12	≤0.015 to >128	NA ^b
	Aztreonam	0.12	64	≤0.015 to >128	76.2
	Meropenem	0.03	0.12	≤0.004 to >8	97.6
	Cefepime	≤0.12	>16	≤0.12 to >16	79.4
	Ceftazidime	0.25	64	≤0.015 to >128	77.2
	Piperacillin-tazobactam	2	64	≤0.25 to >128	84.2
	Amikacin	2	8	≤0.25 to >32	96.6
	Tigecycline	0.5	2	≤0.015 to >8	91.8
	Levofloxacin	0.06	>4	≤0.03 to >4	75.8
MBL negative ^c (23,421)	Aztreonam-avibactam	0.06	0.12	≤0.015 to >128	NA
	Aztreonam	0.12	64	≤0.015 to >128	76.3
	Meropenem	0.03	0.12	≤0.004 to >8	97.9
	Cefepime	≤0.12	>16	≤0.12 to >16	79.7
	Ceftazidime	0.25	64	≤0.015 to >128	77.5
	Piperacillin-tazobactam	2	64	≤0.25 to >128	84.5
	Amikacin	2	8	≤0.25 to >32	96.7
	Tigecycline	0.5	2	≤0.015 to >8	91.8
	Levofloxacin	0.06	>4	≤0.03 to >4	76.0
MBL positive ^d (95)	Aztreonam-avibactam	0.12	1	≤0.015 to 4	NA
	Aztreonam	32	>128	≤0.015 to >128	36.8
	Meropenem	>8	>8	0.25 to >8	10.5
	Cefepime	>16	>16	≤0.12 to >16	4.2
	Ceftazidime	>128	>128	0.25 to >128	4.2
	Piperacillin-tazobactam	>128	>128	0.5 to >128	12.6
	Amikacin	16	>32	1 to >32	61.1
	Tigecycline	1	4	0.06 to >8	88.4
	Levofloxacin	>4	>4	0.06 to >4	36.8
2014–2015					
All (27,836)	Aztreonam-avibactam	0.06	0.12	≤0.015 to 64	NA
	Aztreonam	0.12	64	≤0.015 to >128	75.9
	Meropenem	0.06	0.12	≤0.004 to >8	96.7
	Cefepime	≤0.12	>16	≤0.12 to >16	78.7
	Ceftazidime	0.25	64	≤0.015 to >128	76.8
	Piperacillin-tazobactam	2	64	≤0.25 to >128	85.0
	Amikacin	2	8	≤0.25 to >32	96.7
	Tigecycline	0.5	2	≤0.015 to >8	95.0
	Levofloxacin	0.12	>4	≤0.03 to >4	76.3
MBL negative (27,664)	Colistin ^e	1	>4	≤0.12 to >4	83.2
	Aztreonam-avibactam	0.06	0.12	≤0.015 to 64	NA
	Aztreonam	0.12	64	≤0.015 to >128	76.3
	Meropenem	0.06	0.12	≤0.004 to >8	97.3
	Cefepime	≤0.12	>16	≤0.12 to >16	79.1
	Ceftazidime	0.25	64	≤0.015 to >128	77.3
	Piperacillin-tazobactam	2	64	≤0.25 to >128	85.5
	Amikacin	2	8	≤0.25 to >32	97.0
	Tigecycline	0.5	2	≤0.015 to >8	95.1
MBL positive (172)	Levofloxacin	0.12	>4	≤0.03 to >4	76.6
	Colistin	1	>4	≤0.12 to >4	83.2
	Aztreonam-avibactam	0.12	0.5	≤0.015 to 8	NA
	Aztreonam	64	>128	≤0.015 to >128	25.0
	Meropenem	>8	>8	0.25 to >8	4.1
	Cefepime	>16	>16	0.5 to >16	5.2
	Ceftazidime	>128	>128	32 to >128	0
	Piperacillin-tazobactam	>128	>128	4 to >128	2.3
	Amikacin	16	>32	0.5 to >32	55.8
Tigecycline	1	4	0.12 to 8	89.5	
	Levofloxacin	>4	>4	≤0.03 to >4	24.4
	Colistin	1	>4	0.25 to >4	87.8

^aMICs were interpreted according to CLSI breakpoints (16), with the exception of those for aztreonam-avibactam, for which clinical breakpoints have not yet been assigned; tigecycline, for which MIC interpretative criteria published by the U.S. FDA were used (33); and colistin, for which EUCAST breakpoints were applied (34).

^bNA, not available (no breakpoint criteria have been defined for aztreonam-avibactam).

^cMBL negative, no gene encoding a metallo- β -lactamase included in the screening algorithm was detected by PCR. This isolate type includes carbapenem-sensitive and carbapenem-nonsusceptible metallo- β -lactamase-negative isolates.

^dMBL positive, a gene encoding a metallo- β -lactamase was detected by PCR. This isolate type can also carry one or more additional serine β -lactamases.

^eColistin was tested without added surfactant; data for colistin are for isolates collected in 2014 and 2015 only.

^f*n*, number of isolates tested.

TABLE 4 Comparative MICs of aztreonam-avibactam and aztreonam against 267 isolates of *Enterobacteriaceae* positive for an MBL gene alone or positive for an MBL gene and one or more additional β -lactamase genes

Group (n) ^a	MIC ^b (μ g/ml) for:					
	Aztreonam-avibactam			Aztreonam		
	50%	90%	Range	50%	90%	Range
All MBL producers (267)	0.12	1	≤ 0.015 to 8	64	>128	≤ 0.015 to >128
MBL only (6) ^c	—	—	≤ 0.015 to 0.06	—	—	≤ 0.015 to 1
MBL + OSBL (24)	0.12	0.25	0.03 to 0.5	0.25	2	0.06 to 64
MBL + ESBL (26)	0.25	0.25	0.03 to 0.25	64	>128	0.06 to >128
MBL + ESBL + OSBL (69)	0.12	0.25	≤ 0.015 to 0.5	128	>128	2 to >128
MBL + AmpC (33)	0.12	2	≤ 0.015 to 8	0.25	64	≤ 0.015 to 128
MBL + AmpC + OSBL (28)	0.5	2	≤ 0.015 to 8	16	128	≤ 0.015 to >128
MBL + ESBL + AmpC (13)	0.25	0.25	0.03 to 1	64	>128	1 to >128
MBL + ESBL + AmpC + OSBL (30)	0.12	0.5	≤ 0.015 to 2	128	>128	0.5 to >128
MBL + KPC (1)	—	—	0.5	—	—	>128
MBL + KPC + ESBL (2)	—	—	0.5 to 1	—	—	>128
MBL + KPC + ESBL + OSBL (2)	—	—	0.5 to 2	—	—	>128
MBL + KPC + AmpC + OSBL (1)	—	—	0.5	—	—	>128
MBL + KPC + ESBL + AmpC + OSBL (2)	—	—	0.5	—	—	>128
MBL + OXA-48-like + OSBL (5) ^d	—	—	0.12 to 2	—	—	0.25 to 1
MBL + OXA-48 + ESBL (3)	—	—	0.12 to 0.25	—	—	>128
MBL + OXA-48-like + ESBL + OSBL (13) ^d	0.25	0.5	0.12 to 1	>128	>128	128 to >128
MBL + OXA-48 + AmpC (3)	—	—	0.25 to 1	—	—	0.25 to 16
MBL + OXA-48 + AmpC + OSBL (5)	—	—	0.25	—	—	32
MBL + OXA-48 + ESBL + AmpC + OSBL (1)	—	—	0.25	—	—	128

^aThe MBLs included NDM (142 isolates), VIM (96 isolates), and IMP (29 isolates); the ESBLs included SHV, CTX-M, VEB, and the endogenous ESBL common to *K. oxytoca*. OSBL, original-spectrum β -lactamase. Original-spectrum β -lactamases are enzymes that do not hydrolyze expanded-spectrum cephalosporins or carbapenems and include TEM-1, SHV-1, and SHV-11. n, number of isolates tested.

^b—, MIC₅₀s and MIC₉₀s were not calculated when there were <10 isolates.

^cDoes not include species with endogenous AmpC or ESBL enzymes.

^dIncludes isolates carrying OXA-48 and OXA-232.

apeutic options are currently very limited (17, 18). A phase 2 study to determine the pharmacokinetics, safety, and tolerability of aztreonam-avibactam for the treatment of complicated intra-abdominal infections in hospitalized patients is under way (19). Use of the combination of aztreonam-avibactam presents a novel approach to the treatment of infections caused by pathogens containing multiple β -lactamases, including isolates carrying MBLs, and may serve to address the need for new antimicrobial agents with activity against these carbapenem-nonsusceptible isolates of Gram-negative bacilli (17, 20, 21).

In the current study, aztreonam-avibactam demonstrated potent *in vitro* activity (MIC₉₀, 0.12 μ g/ml) against >50,000 clinical isolates of *Enterobacteriaceae* collected in 40 countries worldwide between 2012 and 2015. Compared with the potency of aztreonam alone, the addition of avibactam resulted in a ≥ 128 -fold improvement in potency for aztreonam-avibactam against isolates that were meropenem nonsusceptible due to the presence of an MBL or other mechanisms of carbapenem resistance (e.g., the production of a KPC). No regional differences in the activity of aztreonam-avibactam were observed, and the activity was maintained against isolates collected over the 4 years surveyed. These data demonstrate the ability of avibactam to restore the activity of aztreonam against *Enterobacteriaceae*.

Previous studies have also demonstrated that avibactam improved the potency of aztreonam against *Enterobacteriaceae* isolates that produced MBLs as well as class A (KPC), class C (AmpC), and class D (OXA-48) β -lactamases (2, 3, 9, 12, 14, 22–26). The current data set extended the observations made previously from studies using smaller collections of isolates (14, 27). A study performed by investigators in China demonstrated that avibactam potentiated the activity of aztreonam against 193 *Enterobacteriaceae* isolates, including isolates with ESBLs and stably derepressed AmpC β -lactamases, and generated a MIC₉₀ of 0.25 μ g/ml, with MICs ranging from ≤ 0.015 to ≥ 128 μ g/ml (22). In the same study, 26 isolates of meropenem-nonsusceptible *Enterobacteriaceae*, including isolates with NDM and IMP MBLs, were tested, and they

generated an aztreonam-avibactam MIC₉₀ of 2 µg/ml and a MIC range of ≤0.06 to 2 µg/ml (22). A European study reported that all 339 *Enterobacteriaceae* isolates tested had aztreonam-avibactam MICs of ≤4 µg/ml (23). In another study, for 65 *Enterobacteriaceae* isolates that were carbapenem resistant by serine carbapenemase production, MBL production, or porin loss plus AmpC or ESBL production, the MIC range for aztreonam-avibactam was ≤0.03 to 4 µg/ml (3). Avibactam was reported to lower the aztreonam MICs of AmpC-, ESBL-, and KPC-producing *Enterobacteriaceae* by up to 2,048-fold, to a maximum MIC of 4 µg/ml (2). Aztreonam-avibactam also demonstrated potent *in vitro* activity (MIC₉₀, 0.5 µg/ml; MIC range, ≤0.06 to 4 µg/ml) against 133 porin-deficient, carbapenem-nonsusceptible *Enterobacteriaceae* isolates from northern France (24). Lastly, aztreonam-avibactam inhibited all isolates in a collection of 177 (172 *Enterobacteriaceae* isolates and 5 *P. aeruginosa* isolates) carbapenemase-producing Gram-negative bacilli (108 KPC, 32 NDM, 11 IMP, 8 OXA-48, 4 OXA-181, 2 OXA-232, 5 IMI, 4 VIM, and 3 SME producers) from the United States and Singapore at a concentration of 16 µg/ml (MIC₉₀, 1 µg/ml) (25).

In this study, 25 isolates of MBL-negative *Enterobacteriaceae* with aztreonam-avibactam MICs of ≥16 µg/ml and meropenem MICs ranging from 0.03 to >8 µg/ml were detected (9 isolates in 2012, 4 in 2013, 5 in 2014, and 7 in 2015). The mechanism responsible for the increased MICs of aztreonam-avibactam in these 25 isolates remains unclear and may represent a combination of factors. For 16 isolates, the mechanism(s) appears to be aztreonam related rather than avibactam related, because those isolates remained susceptible to ceftazidime-avibactam. It is possible that some of the isolates may harbor mutations in PBP3, such as the 4-amino-acid insertion shown to reduce the activity of aztreonam-avibactam (28). Resistance may also arise from single amino acid substitutions or Ω-loop mutations in coresident class A and C serine β-lactamases that can lead to a reduced affinity of avibactam for the β-lactamase binding site, resulting in poor inhibition of β-lactamase activity (13, 29, 30). Porin deficits and overexpression of efflux pumps may also be contributory. Other investigators have shown that variants of KPC-2 can be resistant to avibactam by mutations in residue S130, as well as residues K234 and R220, which are important residues for avibactam binding to and inactivation of KPC-2 (31).

The *in vitro* activities of aztreonam-avibactam and aztreonam alone were less potent (MIC₉₀, 32 µg/ml) against *P. aeruginosa* than against *Enterobacteriaceae*, and the addition of avibactam to aztreonam did not improve the potency of aztreonam, suggesting that resistance to aztreonam in *P. aeruginosa* is, at least in part, a function of mechanisms other than β-lactamases, such as upregulation of the MexAB-OprM efflux transport system (32). Other investigators have also observed that the addition of avibactam to aztreonam did not generate an improvement in the potency of aztreonam against clinical isolates of *P. aeruginosa* (22). The distributions of the MICs of aztreonam-avibactam for MBL-negative and MBL-positive *P. aeruginosa* isolates have been shown to be similar to those for meropenem-susceptible and meropenem-nonsusceptible populations, respectively (9). The treatment of patients with infections due to carbapenem-resistant nonfermentative Gram-negative bacilli remains difficult because of the MDR profiles of many of these isolates.

The clinical development of aztreonam-avibactam appears to be well justified, as it may be a valuable alternative for the treatment of infections caused by isolates of the *Enterobacteriaceae* that are carbapenem nonsusceptible, especially infections caused by MBL-producing pathogens, as infections caused by these pathogens continue to emerge worldwide. Infection control and antimicrobial stewardship practices will remain important to maintaining the low prevalence of mobile carbapenemase genes and to preserving the activities of all antimicrobial agents. As β-lactam-avibactam combinations are introduced into clinical use, ongoing surveillance for β-lactamase-mediated resistance and other mechanisms of resistance is imperative to ensure the long-term viability and effectiveness of these and other antimicrobial agents. It will also be important to monitor for and characterize nonsusceptible isolates and to correlate clinical outcomes with identified resistance genotypes.

MATERIALS AND METHODS

Bacterial isolates. Isolates were collected from 2012 to 2015 from multiple sites around the world, including 208 medical center laboratories in 40 countries. The sites were requested each year to collect predefined quotas of clinical isolates of selected bacterial species (limited to one isolate per patient per annum) from hospitalized patients with specific infection types (intra-abdominal, urinary tract, skin and soft tissue, lower respiratory, and bloodstream infections) and to submit them to a central laboratory (International Health Management Associates, Inc. [IHMA], Schaumburg, IL, USA) for confirmatory identification and reference antimicrobial susceptibility testing. In total, from 2012 to 2015, 51,352 *Enterobacteriaceae* isolates and 11,842 *P. aeruginosa* isolates were collected, their identities were confirmed, and reference antimicrobial susceptibility testing was performed. Because quotas of selected bacterial species were used for the collection of isolates, this study was not structured to evaluate the prevalence of organisms causing specific infections or resistance phenotypes. The identities of all isolates were confirmed using matrix-assisted laser desorption/ionization–time of flight spectrometry (Bruker Daltonics, Billerica, MA, USA). All components of this study were coordinated by IHMA, including the development and management of the central study database.

Isolates submitted by medical center laboratories were grouped by country into five major geographical regions: Asia/South Pacific (Australia, China, Hong Kong, Japan, Malaysia, Philippines, South Korea, Taiwan, Thailand), Europe (Austria, Belgium, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Poland, Portugal, Romania, Russia, Spain, Sweden, Turkey, United Kingdom), Latin America (Argentina, Brazil, Chile, Colombia, Mexico, Venezuela), Middle East/Africa (Israel, Kenya, Kuwait, Nigeria, South Africa), and North America (United States). Of the 51,352 *Enterobacteriaceae* isolates included in this study, 17.8% ($n = 9,149$) were from the Asia/South Pacific region, 48.3% ($n = 24,826$) were from Europe, 14.9% ($n = 7,665$) were from Latin America, 8.2% ($n = 4,232$) were from the Middle East/Africa region, and 10.7% ($n = 5,480$) were from North America. The composition of the *Enterobacteriaceae* isolates collected in each region, by species, is shown in the Table S1 in the supplemental material. Of the 11,842 *P. aeruginosa* isolates included in this study, 17.2% ($n = 2,038$) were from the Asia/South Pacific region, 48.4% ($n = 5,728$) were from Europe, 15.1% ($n = 1,794$) were from Latin America, 8.1% ($n = 964$) were from the Middle East/Africa region, and 11.1% ($n = 1,318$) were from North America.

The composition of the *Enterobacteriaceae* isolates by specimen source was as follows: intra-abdominal, $n = 10,025$ (19.5% of all isolates tested); urinary tract, $n = 14,604$ (28.4%); skin and soft tissue, $n = 12,698$ (24.7%); lower respiratory tract, $n = 11,426$ (22.3%); bloodstream, $n = 2,458$ (4.8%); and other, $n = 141$ (0.3%). The composition of the *P. aeruginosa* isolates by specimen source was as follows: intra-abdominal, $n = 1,171$ (9.9% of all isolates tested); urinary tract, $n = 1,696$ (14.3%); skin and soft tissue, $n = 3,521$ (29.7%); lower respiratory tract, $n = 5,052$ (42.7%); bloodstream, $n = 387$ (3.3%); and other, $n = 15$ (0.1%).

Antimicrobial susceptibility testing. All antimicrobial susceptibility testing was performed at IHMA using in-house-prepared broth microdilution panels produced, inoculated, incubated, interpreted, and quality controlled by standardized CLSI methods (15, 16). Avibactam was tested at a fixed concentration of 4 $\mu\text{g/ml}$ in combination with 2-fold dilutions of aztreonam. MICs were interpreted using CLSI breakpoints (16) for all antimicrobial agents except for those for which CLSI breakpoints are not available: aztreonam-avibactam, for which clinical breakpoints have not yet been assigned; tigecycline, for which U.S. FDA MIC breakpoints were used (33); and colistin, for which EUCAST MIC breakpoints (34) were applied.

Isolates of *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis* were phenotypically screened for possible ESBL production using ceftazidime and aztreonam MICs ($>1 \mu\text{g/ml}$) and confirmed to be ESBL producers by testing with clavulanate in combination with ceftazidime and cefotaxime, following CLSI guidelines (16). If the MICs of the screening β -lactams exceeded the highest dilution on the broth microdilution panel, confirmation of ESBL production was determined using disk diffusion testing (16).

Data analysis included grouping of the *Enterobacteriaceae* isolates into MBL-negative and MBL-positive isolates. An MBL-negative isolate was defined as an isolate for which molecular testing (i.e., PCR [described below]) did not detect the presence of an MBL gene. MBL-negative isolates included isolates that were susceptible to all carbapenems tested, isolates whose genomes encoded serine carbapenemases or other serine β -lactamases, and isolates that were resistant to carbapenems by virtue of nonenzymatic mechanisms, such as reduced outer membrane permeability. An MBL-positive isolate was defined as an isolate for which PCR detected the presence of one of the MBL genes (IMP, VIM, NDM, GIM, and SPM) that was included in the screening algorithm. These isolates could also contain one or more additional serine β -lactamases.

Molecular identification of β -lactamase genes in *Enterobacteriaceae* and *P. aeruginosa*. *Enterobacteriaceae* isolates were chosen for PCR testing to detect the presence of β -lactamase genes when they met at least one of the following criteria: (i) the isolates demonstrated a positive result for ESBL production by a confirmatory test, (ii) the isolates demonstrated a negative result for ESBL production by a confirmatory test but were resistant to ceftazidime (MIC, $\geq 16 \mu\text{g/ml}$), and (iii) the isolates had meropenem, doripenem, or imipenem MICs of $\geq 2 \mu\text{g/ml}$ (nonsusceptible) (isolates of *Proteaeae* with imipenem MICs of 2 or 4 $\mu\text{g/ml}$ but meropenem and doripenem MICs of $<2 \mu\text{g/ml}$ were excluded) (16). A multiplex PCR assay followed by DNA sequencing was performed as previously described (35) to molecularly characterize β -lactamase genes encoding MBLs (i.e., IMP, VIM, NDM, GIM, and SPM) and other β -lactamases (i.e., SHV, TEM, CTX-M, VEB, PER, GES, ACC, CMY, DHA, FOX, ACT, MIR, MOX, KPC, and OXA-48-like). *P. aeruginosa* isolates with meropenem, doripenem, or imipenem MICs of $\geq 4 \mu\text{g/ml}$ (nonsusceptible) were screened for the presence of β -lactamase genes encoding MBLs (IMP, VIM, NDM,

GIM, and SPM) and other β -lactamases (SHV, TEM, VEB, PER, GES, KPC, and OXA-24-like) as previously described (36).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00472-17>.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

This study was sponsored by AstraZeneca Pharmaceuticals LP, which also included compensation fees for manuscript preparation. AstraZeneca's rights to aztreonam-avibactam were acquired by Pfizer in December 2016.

Medical writing and editorial support were provided by J.A.K., a consultant for IHMA and an employee of the University of Manitoba and Diagnostic Services Manitoba, and K.M.K., an employee of IHMA. All authors provided analysis input and have read and approved the final manuscript.

M.A.H. and D.F.S. are employees of IHMA. None of the IHMA authors or J.A.K. has a personal financial interest in the sponsor of this paper (AstraZeneca Pharmaceuticals). B.L.M.D.J. was an employee of and shareholder in AstraZeneca at the time of the study and is currently an employee of Pfizer. P.A.B. is a former employee of AstraZeneca.

We gratefully acknowledge the contributions of the study investigators, laboratory personnel, and all members of the AstraZeneca Surveillance program.

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