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In Vitro Susceptibility of Global Surveillance Isolates of *Pseudomonas aeruginosa* to Ceftazidime-Avibactam (INFORM 2012 to 2014)

Wright W. Nichols,[a]* Boudewijn L. M. de Jonge,[a] Krystyna M. Kazmierczak,[b] James A. Karlowsky,[b] Daniel F. Sahm[b]

AstraZeneca Pharmaceuticals, Waltham, Massachusetts, USA[a]; International Health Management Associates, Inc., Schaumburg, Illinois, USA[b]

Broth microdilution antimicrobial susceptibility testing was performed for ceftazidime-avibactam and comparator agents against 7,062 clinical isolates of *Pseudomonas aeruginosa* collected from 2012 to 2014 in four geographic regions (Europe, Asia/South Pacific, Latin America, Middle East/Africa) as part of the International Network for Optimal Resistance Monitoring (INFORM) global surveillance program. The majority of isolates were susceptible to ceftazidime-avibactam, with the proportions susceptible differing marginally across the four regions (MIC_{90}, 8 to 16 μg/ml; 88.7 to 93.2% susceptible), in contrast to lower susceptibilities to the following comparator β-lactam agents: ceftazidime (MIC_{90}, 32 to 64 μg/ml; 71.5 to 80.8% susceptible), meropenem (MIC_{90}, >8 μg/ml; 64.9 to 77.4% susceptible), and piperacillin-tazobactam (MIC_{90}, >128 μg/ml; 62.3 to 71.3% susceptible). Compared to the overall population, susceptibility to ceftazidime-avibactam of isolates that were nonsusceptible to ceftazidime ($n = 1,627$) was reduced to between 56.8% (Middle East/Africa; MIC_{90}, 64 μg/ml) and 68.9% (Asia/South Pacific; MIC_{90}, 128 μg/ml), but these percentages were higher than susceptibilities to other β-lactam agents (0 to 44% susceptible, depending on region and agent; meropenem MIC_{90}, >8 μg/ml; 26.5 to 43.9% susceptible). For this subset of isolates, susceptibilities to amikacin (MIC_{90}, >32 μg/ml; 53.2 to 80.0% susceptible) and colistin (MIC_{90}, 1 μg/ml; 98.5 to 99.5% susceptible) were comparable to or higher than that of ceftazidime-avibactam. A similar observation was made with isolates that were nonsusceptible to meropenem ($n = 1,926$), with susceptibility to ceftazidime-avibactam between 67.8% (Middle East/Africa; MIC_{90}, 64 μg/ml) and 74.2% (Europe; MIC_{90}, 32 μg/ml) but again with reduced susceptibility to comparators except for amikacin (MIC_{90}, >32 μg/ml; 56.8 to 78.7% susceptible) and colistin (MIC_{90}, 1 μg/ml; 98.9 to 99.3% susceptible). Of the 8% of isolates not susceptible to ceftazidime-avibactam, the nonsusceptibility of half could be explained by their possession of genes encoding metallo-β-lactamases. The data reported here are consistent with results from other country-specific and regional surveillance studies and show that ceftazidime-avibactam demonstrates *in vitro* activity against globally collected clinical isolates of *P. aeruginosa*, including isolates that are resistant to ceftazidime and meropenem.

Ceftazidime has been an important antibacterial agent in the treatment of infections caused by *Pseudomonas aeruginosa* since the early 1980s (1, 2). However, mutational resistance can develop by stable derepression of the normally inducible chromosomally encoded AmpC β-lactamase (3), and by other non-β-lactamase-mediated mutations (4, 5). In addition, exogenously acquired resistance through the acquisition of genes encoding β-lactamases, such as bla_{VIM-2} (6), has also been reported, though it is less common than mutational resistance (4, 7). As a result, the effectiveness of ceftazidime against *P. aeruginosa* has significantly eroded (4–7).

Avibactam is a non-β-lactam β-lactamase inhibitor that inhibits class A, class C, and some class D β-lactamases (8). Among the class C β-lactamases, avibactam inhibits the AmpC enzyme of *P. aeruginosa* (8, 9). When avibactam is combined with ceftazidime, this inhibition results in the restoration of ceftazidime activity against *P. aeruginosa* isolates that are resistant to ceftazidime through stably derepressed synthesis of the AmpC enzyme, regardless of the allelic variant of bla_{AmpC} carried (10, 11). The inhibition by avibactam of the *P. aeruginosa* AmpC β-lactamase *in vitro* restores not only low MICs of ceftazidime against that organism but also bactericidal activity at those lower concentrations (12). The restoration of *in vitro* activity translates to restoration of ceftazidime efficacy against ceftazidime-resistant *P. aeruginosa in vivo* (13–15). The restoration by avibactam of the *in vitro* activity of ceftazidime described above has extended to collections of unselected clinical isolates of *P. aeruginosa* in surveillance studies (16–24).

Ceftazidime-avibactam has been approved by the United States Food and Drug Administration (U.S. FDA) for the treatment of complicated intraabdominal infections and complicated urinary tract infections caused by *P. aeruginosa* and other Gram-negative species in patients with limited or no alternative treatment options (25). Surveillance studies are an important tool to monitor the activity of agents on a local and global level and to detect the possible emergence of resistance that might compromise therapy, as has been observed for ceftazidime-avibactam *in vitro* (13, 26). In the present work, the activity of ceftazidime-avibactam against *P. aeruginosa* was assessed through the Interna-

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Address correspondence to Krystyna M. Kazmierczak, kkazmierczak@ihmainc.com.

* Present address: Wright W. Nichols, Cambridge, Massachusetts, USA.

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tional Network for Optimal Resistance Monitoring (INFORM) global surveillance study across four regions for 3 years (2012 to 2014), by (i) comparing the relative *in vitro* activities of ceftazidime and ceftazidime-avibactam, in order to understand what extra proportion of susceptible isolates is provided by the inhibition of β-lactamases by avibactam *in vitro*; (ii) examining the potential clinical value that ceftazidime-avibactam might represent based on its *in vitro* activity compared to the activities of other important antipseudomonal agents, such as meropenem, imipenem, doripenem, cefepime, piperacillin-tazobactam, amikacin, and colistin; and (iii) placing this study of recent clinical isolates into context with other recent surveillance studies (16–24) and studies that were confined to subpopulations of isolates of *P. aeruginosa* selected as being resistant to ceftazidime or other β-lactams (27–29).

(Parts of this research were presented at the 24th, 25th, and 26th European Congresses of Clinical Microbiology and Infectious Diseases [30–32] and the 54th Interscience Conference on Antimicrobial Agents and Chemotherapy [33].)

MATERIALS AND METHODS

***P. aeruginosa* isolates.** From 2012 to 2014, the INFORM global surveillance program collected 7,062 isolates of *P. aeruginosa* from medical center laboratories in Europe (19 countries, 93 laboratories), Asia/South Pacific (9 countries, 41 laboratories), Latin America (6 countries, 26 laboratories), and the Middle East/Africa (5 countries, 16 laboratories). All isolates were shipped to a central reference laboratory, International Health Management Associates, Inc. (IHMA; Schaumburg, IL, USA), where their identities were confirmed as *P. aeruginosa* by the use of MALDI-TOF (matrix-assisted laser desorption ionization-time of flight) spectrometry (Biotyper instrument; Bruker Daltonics, Billerica, MA, USA).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed following the Clinical and Laboratory Standards Institute (CLSI) standard method for broth microdilution using in-house-prepared, 96-well panels (34, 35). Avibactam was tested at a fixed concentration of 4 μg/ml in combination with doubling dilutions of ceftazidime (25). Colistin was tested with a final concentration of 0.002% polysorbate 80 in each panel well (36). MICs of all agents except ceftazidime-avibactam were interpreted using CLSI breakpoints (34). Ceftazidime-avibactam MICs were interpreted using U.S. FDA MIC breakpoints for *P. aeruginosa* (25) (susceptible, ≤8 μg/ml; resistant, ≥16 μg/ml) because the CLSI currently does not publish MIC interpretative breakpoints for this agent.

Screening isolates for β-lactamase genes. All meropenem-, imipenem-, and doripenem-nonsusceptible isolates were screened for the presence of genes encoding carbapenemases (KPC, OXA-24 family, GES, VIM, IMP, NDM, SPM, GIM) and selected extended-spectrum β-lactamases (ESBLs; SHV, TEM, VEB, PER, GES) using Qiagen's multiplex PCR kit (Valencia, CA) according to the manufacturer's recommendations and published primer sets (37, 38). The genes detected were sequenced and compared to sequences in publically available databases (www.ncbi.nlm.nih.gov; www.lahey.org).

RESULTS

Antimicrobial susceptibility testing results for ceftazidime-avibactam and comparator agents are shown in Table 1. The susceptibility to ceftazidime-avibactam was 92.0% overall, with slight variations (88.7 to 93.2%) across the surveyed geographic regions, and exceeded the susceptibilities to all other β-lactams tested, including carbapenems (57.2 to 78.5% susceptible, depending on compound and region). Susceptibility to ceftazidime-avibactam was comparable or greater than that to amikacin (81.8 to 94.4% sus-

TABLE 1 *In vitro* activities of ceftazidime-avibactam and comparator agents tested against 7,062 *P. aeruginosa* isolates collected in 2012 to 2014 from patients in four geographic regions

Region (no. of isolates)	Antimicrobial agent[a]	MIC_{90} (μg/ml)	% Susceptible[b]
All (7,062)	Ceftazidime-avibactam	8	92.0
	Ceftazidime	64	77.0
	Cefepime	>16	78.3
	Piperacillin-tazobactam	>128	68.6
	Doripenem	>4	74.3
	Meropenem	>8	72.7
	Imipenem	>8	61.4
	Colistin	1	99.5
	Amikacin	32	89.4
	Levofloxacin	>4	71.9
Europe (3,893)	Ceftazidime-avibactam	8	92.6
	Ceftazidime	64	77.4
	Cefepime	16	78.8
	Piperacillin-tazobactam	>128	69.4
	Doripenem	>4	74.4
	Meropenem	>8	72.9
	Imipenem	>8	60.3
	Colistin	1	99.5
	Amikacin	32	89.7
	Levofloxacin	>4	71.3
Asia/South Pacific (1,392)	Ceftazidime-avibactam	8	93.2
	Ceftazidime	64	78.1
	Cefepime	16	80.2
	Piperacillin-tazobactam	>128	71.3
	Doripenem	>4	78.5
	Meropenem	>8	77.4
	Imipenem	>8	67.0
	Colistin	1	99.5
	Amikacin	8	94.4
	Levofloxacin	>4	77.2
Latin America (1,088)	Ceftazidime-avibactam	16	88.7
	Ceftazidime	64	71.5
	Cefepime	>16	73.2
	Piperacillin-tazobactam	>128	62.3
	Doripenem	>4	66.8
	Meropenem	>8	64.9
	Imipenem	>8	57.2
	Colistin	1	99.4
	Amikacin	>32	81.8
	Levofloxacin	>4	64.7
Middle East/Africa (689)	Ceftazidime-avibactam	8	91.7
	Ceftazidime	32	80.8
	Cefepime	16	79.5
	Piperacillin-tazobactam	>128	68.8
	Doripenem	>4	77.1
	Meropenem	>8	74.8
	Imipenem	>8	63.3
	Colistin	1	99.6
	Amikacin	16	90.1
	Levofloxacin	>4	75.9

[a] Colistin was tested in the presence of a final concentration of 0.002% polysorbate 80.
[b] Values are based on CLSI breakpoints, except for ceftazidime-avibactam, for which FDA breakpoints were used.

ceptible). Colistin was the most active agent tested, with over 99% of the isolates susceptible to this agent. The MIC_{90}s for ceftazidime-avibactam were 8 μg/ml for isolates from Europe, Asia/South Pacific, and Middle East/Africa and 16 μg/ml (with 88.7% of isolates susceptible) for isolates from Latin America, where susceptibilities to other agents were also generally lower except for that to colistin. The MIC_{90} for ceftazidime-avibactam was consistently two to three doubling dilutions lower than the MIC_{90} for ceftazidime in all four geographic regions (Tables 1 and 2).

Of the 7,062 isolates of *P. aeruginosa* collected, 2,840 were nonsusceptible to one or more of the carbapenems tested and were molecularly characterized for the presence of relevant β-lactamase genes (Table 3). Three hundred six isolates (10.8% of this nonsusceptible subpopulation, 4.3% of all isolates) were identified as metallo-β-lactamase (MBL) positive (269 VIM type, 34 IMP type, 3 NDM-1), with 21 of these cocarrying an ESBL, serine carbapenemase, or TEM type original-spectrum β-lactamase (OSBL). In addition, 76 MBL-negative isolates that encoded serine carbapenemases were identified (KPC-2, GES-2, GES-5, GES-6, GES-13), as well as 16 that carried GES ESBLs and carbapenemases (GES-1 and GES-5; GES-19 and GES-20), 70 that encoded ESBLs (SHV, VEB, PER, and GES type), 1 that carried a GES with an undefined spectrum of activity, and 11 that carried only a TEM-type OSBL. In 2,360 isolates (83.1% of the carbapenem-nonsusceptible subpopulation, 33.4% of all isolates), no acquired β-lactamase gene could be detected, and carbapenem nonsusceptibility in most of these isolates was likely due to porin defects and/or upregulated efflux in combination with overexpression of chromosomal AmpC (39). Alternatively, some might also contain β-lactamases that were not part of the screening algorithm. Most metallo-β-lactamase-positive isolates (291/306; 95.1%) were resistant to ceftazidime-avibactam, which was expected since avibactam does not inhibit these types of enzymes (Table 3) (40). For the remainder of the isolates (i.e., those without detected metallo-β-lactamase genes, $n = 6,756$), 96.0% were susceptible to ceftazidime-avibactam, compared to only 80.3% for ceftazidime (Table 2).

A total of 563 ceftazidime-avibactam-resistant isolates were identified in this study (8% of all isolates). Of these, 291 (51.7%) were metallo-β-lactamase positive, 21 isolates carried genes for serine carbapenemases (KPC-2, GES type) with or without ESBLs, 1 isolate carried a GES of undefined activity, and 51 isolates harbored only ESBLs, i.e., SHV-5 (1 isolate), VEB type (25 isolates), PER type (13 isolates), or GES type (12 isolates). No acquired β-lactamase gene could be identified in the remaining 199 ceftazidime-avibactam-resistant isolates (Table 3).

The percentage of isolates from each geographic region that tested as nonsusceptible to ceftazidime ranged from 19.2 to 28.5% (Table 1). Between 56.8 and 68.9% of these ceftazidime-nonsusceptible *P. aeruginosa* isolates remained susceptible to ceftazidime-avibactam, the highest percentage compared to all other β-lactams tested (cefepime, <22% susceptible; piperacillin-tazobactam, <8%; carbapenems, <44%) (Table 4). This subset of isolates showed comparable or higher susceptibilities to colistin and amikacin in all regions but Latin America, where only 53.2% of isolates remained susceptible to amikacin. Table S1 in the supplemental material demonstrates that the MIC_{90} of ceftazidime-avibactam for ceftazidime-nonsusceptible, metallo-β-lactamase-negative isolates was at least two to three doubling dilutions lower

TABLE 2 Cumulative MIC distributions for ceftazidime and ceftazidime-avibactam against 7,062 clinical isolates of *P. aeruginosa* in 2012 to 2014 in four geographic regions

Isolate type or region (no. of isolates)	Antimicrobial agent	No. (cumulative %) of isolates inhibited at an MIC (μg/ml) of[a]:								
		≤0.5	1	2	4	8	16	32	64	128	>128
All isolates (7,062)	Ceftazidime	93 (1.3)	606 (9.9)	2,805 (49.6)	1,295 (68.0)	636 (77.0)	320 (81.5)	479 (88.3)	**452 (94.7)**	190 (97.4)	186 (100)
	Ceftazidime-avibactam	167 (2.4)	1,178 (19.0)	3,241 (64.9)	1,159 (81.4)	**754 (92.0)**	176 (94.5)	163 (96.8)	115 (98.5)	47 (99.1)	62 (100)
MBL[b] negative (6,756)[c]	Ceftazidime	93 (1.4)	606 (10.3)	2,803 (51.8)	1,295 (71.0)	630 (80.3)	285 (84.5)	**390 (90.3)**	364 (95.7)	163 (98.1)	127 (100)
	Ceftazidime-avibactam	167(2.5)	1,178 (19.9)	3,238 (67.8)	1,159 (85.0)	**742 (96.0)**	130 (97.9)	68 (98.9)	43 (99.5)	15 (99.8)	16 (100)
Europe (3,893)	Ceftazidime	60 (1.5)	361 (10.8)	1,542 (50.4)	711 (68.7)	339 (77.4)	178 (82.0)	272 (89.0)	**255 (95.5)**	94 (97.9)	81 (100)
	Ceftazidime-avibactam	106 (2.7)	710 (21.0)	1,759 (66.1)	635 (82.5)	**395 (92.6)**	99 (95.1)	92 (97.5)	58 (99.0)	18 (99.5)	21 (100)
Asia/South Pacific (1,392)	Ceftazidime	16 (1.1)	102 (8.5)	591 (50.9)	251 (69.0)	127 (78.1)	51 (81.8)	79 (87.4)	**84 (93.5)**	38 (96.2)	53 (100)
	Ceftazidime-avibactam	32 (2.3)	208 (17.2)	691 (66.9)	218 (82.5)	**148 (93.2)**	17 (94.4)	12 (95.3)	21 (96.8)	17 (98.0)	28 (100)
Latin America (1,088)	Ceftazidime	13 (1.2)	86 (9.1)	392 (45.1)	190 (62.6)	97 (71.5)	70 (77.9)	85 (85.8)	**70 (92.2)**	38 (95.7)	47 (100)
	Ceftazidime-avibactam	20 (1.8)	156 (16.2)	469 (59.3)	175 (75.4)	145 (88.7)	**47 (93.0)**	43 (97.0)	17 (98.5)	6 (99.1)	10 (100)
Middle East/Africa (689)	Ceftazidime	4 (0.6)	57 (8.9)	280 (49.5)	143 (70.2)	73 (80.8)	21 (83.9)	**43 (90.1)**	43 (96.4)	20 (99.3)	5 (100)
	Ceftazidime-avibactam	9 (1.3)	104 (16.4)	322 (63.1)	131 (82.1)	**66 (91.7)**	13 (93.6)	16 (95.9)	19 (98.7)	6 (99.6)	3 (100)

[a] MIC_{90}s are in bold.
[b] MBL, metallo-β-lactamase.
[c] This isolate type includes carbapenem-sensitive and carbapenem-nonsusceptible metallo-β-lactamase-negative isolates.

TABLE 3 Occurrence of β-lactamase genes in carbapenem-nonsusceptible *P. aeruginosa* isolates, stratified by phenotype[a]

Parameter, phenotype, and β-lactamase content	Enzyme type	Total no. of isolates	No. of isolates that were:		
			CAZ-NS	CAZ-NS, CAZ-AVI-S	CAZ-NS, CAZ-AVI-R
Total tested		7,062	1,627	1,064	563
Carbapenem NS[b]		2,840	1,257	709	548
MBL positive, with or without serine β-lactamase genes	VIM ± TEM-OSBL	252	244	6	238
	VIM + VEB	6	6		6
	VIM + PER	5	5		5
	VIM + SHV	4	4	1	3
	VIM + GES ESBL-like	1	1		1
	VIM + KPC	1	1		1
	IMP ± TEM-OSBL	34	34		34
	NDM	1	1		1
	NDM + VEB	2	2		2
KPC positive, MBL negative	KPC	28	28	22	6
GES positive, MBL negative	GES carbapenemase	48	39	38	1
	GES carbapenemase + GES ESBL-like	16	16	2	14
	GES ESBL-like	20	20	8	12
	GES spectrum undefined	1	1		1
ESBL positive, MBL negative	VEB ± TEM-OSBL	25	25		25
	PER ± TEM-OSBL	23	22	9	13
	SHV	2	2	1	1
OSBL positive, MBL negative	TEM-OSBL	11	7	7	
No acquired *bla* detected[c]		2,360	799	615	184
Carbapenem S (not characterized)		4,222	370	355	15

[a] CAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; S, susceptible; NS, nonsusceptible; R, resistant.
[b] Isolates nonsusceptible to meropenem, imipenem, or doripenem were screened for β-lactamase (*bla*) genes.
[c] Isolates are presumed to contain the chromosomal *ampC* gene common to *P. aeruginosa*.

than that of ceftazidime against the same isolates in all four geographic regions, with 72.4 to 82.7% of those isolates being susceptible to ceftazidime-avibactam.

The percentage of isolates from each geographic region that were meropenem nonsusceptible ranged from 22.6 to 35.1% (Table 1). In total, there were 1,926 meropenem-nonsusceptible isolates, 84.4% (1,626) of which were metallo-β-lactamase negative. Compared to the findings for the overall population, the percentages of susceptibility to ceftazidime-avibactam in meropenem-nonsusceptible isolates of *P. aeruginosa* decreased only 18 to 24% to between 67.8 and 74.2%, depending on the region, whereas the activities of other β-lactams were much more reduced (cefepime, ≤43% susceptible; piperacillin-tazobactam, <32%; doripenem and imipenem, <10%) (Table 5). The meropenem-nonsusceptible *P. aeruginosa* isolates showed higher percentages of susceptibility to colistin in all regions, whereas the percentages of susceptibility to amikacin were comparable or higher in the Asia/South Pacific and Middle East/Africa regions. The percentage of susceptibility to ceftazidime-avibactam among the meropenem-nonsusceptible isolates was 72.4%, which increased to 84.9% when isolates harboring metallo-β-lactamase genes were excluded, and this trend was apparent across all surveyed regions (see Table S2 in the supplemental material).

DISCUSSION

The activity of ceftazidime-avibactam was assessed against 7,062 *P. aeruginosa* isolates from clinics in four geographic regions over the period 2012 to 2014. The proportion of isolates susceptible to ceftazidime-avibactam was 92.0%, in comparison to 77.0% being susceptible to ceftazidime alone. Hence, avibactam restored susceptibility to ceftazidime for 1,064 of 1,627 ceftazidime-nonsusceptible isolates (65.4%). For the majority of these ceftazidime-nonsusceptible, ceftazidime-avibactam-susceptible isolates (615 out of 709 tested), none of the *bla* genes tested for could be detected, and nonsusceptibility was inferred to result from hyperproduction of the chromosomally encoded AmpC, although no tests were performed to assess stable derepression of *bla* genes or porin expression levels. This would be consistent with the observed activity of ceftazidime-avibactam against *P. aeruginosa* characterized as stably derepressed for production of AmpC (10, 11).

The MIC_{90} for ceftazidime-avibactam was 8 μg/ml for isolates of *P. aeruginosa* from Europe, Asia/South Pacific, and Middle East/Africa and one doubling dilution higher for isolates from Latin America, where the susceptibility rate was 88.7%. These results are in agreement with previous surveillance studies of cefta-

TABLE 4 *In vitro* activities of ceftazidime-avibactam and comparator agents tested against 1,627 ceftazidime-nonsusceptible *P. aeruginosa* isolates collected in 2012 to 2014 from patients in four geographic regions

Region (no. of isolates)	Antimicrobial agent[a]	MIC_{90} (μg/ml)	% Susceptible[b]
All (1,627)	Ceftazidime-avibactam	64	65.4
	Ceftazidime	>128	0
	Cefepime	>16	19.7
	Piperacillin-tazobactam	>128	5.4
	Doripenem	>4	32.4
	Meropenem	>8	31.8
	Imipenem	>8	26.1
	Colistin	1	99.1
	Amikacin	>32	65.7
	Levofloxacin	>4	32.3
Europe (880)	Ceftazidime-avibactam	64	67.3
	Ceftazidime	128	0
	Cefepime	>16	21.6
	Piperacillin-tazobactam	>128	5.6
	Doripenem	>4	30.5
	Meropenem	>8	29.6
	Imipenem	>8	23.2
	Colistin	1	99.5
	Amikacin	>32	66.0
	Levofloxacin	>4	30.8
Asia/South Pacific (305)	Ceftazidime-avibactam	128	68.9
	Ceftazidime	>128	0
	Cefepime	>16	21.0
	Piperacillin-tazobactam	>128	7.2
	Doripenem	>4	42.0
	Meropenem	>8	43.9
	Imipenem	>8	38.4
	Colistin	1	98.7
	Amikacin	>32	80.0
	Levofloxacin	>4	43.0
Latin America (310)	Ceftazidime-avibactam	64	60.3
	Ceftazidime	>128	0
	Cefepime	>16	16.1
	Piperacillin-tazobactam	>128	4.2
	Doripenem	>4	26.8
	Meropenem	>8	26.5
	Imipenem	>8	22.6
	Colistin	1	98.7
	Amikacin	>32	53.2
	Levofloxacin	>4	26.8
Middle East/Africa (132)	Ceftazidime-avibactam	64	56.8
	Ceftazidime	128	0
	Cefepime	>16	12.1
	Piperacillin-tazobactam	>128	3.0
	Doripenem	>4	35.6
	Meropenem	>8	31.8
	Imipenem	>8	25.0
	Colistin	1	98.5
	Amikacin	>32	59.9
	Levofloxacin	>4	31.1

[a] Colistin was tested in the presence of a final concentration of 0.002% polysorbate 80.
[b] Values are based on CLSI breakpoints, except for ceftazidime-avibactam, for which FDA breakpoints were used.

TABLE 5 *In vitro* activities of ceftazidime-avibactam and comparator agents tested against 1,926 meropenem-nonsusceptible *P. aeruginosa* isolates collected in 2012 to 2014 from patients in four geographic regions

Region (no. of isolates)	Antimicrobial agent[a]	MIC_{90} (μg/ml)	% Susceptible[b]
All (1,926)	Ceftazidime-avibactam	64	72.4
	Ceftazidime	128	42.4
	Cefepime	>16	41.0
	Piperacillin-tazobactam	>128	28.4
	Doripenem	>4	9.3
	Meropenem	>8	0
	Imipenem	>8	4.8
	Colistin	1	99.2
	Amikacin	>32	67.4
	Levofloxacin	>4	34.0
Europe (1,056)	Ceftazidime-avibactam	32	74.2
	Ceftazidime	128	41.3
	Cefepime	>16	41.0
	Piperacillin-tazobactam	>128	27.0
	Doripenem	>4	9.8
	Meropenem	>8	0
	Imipenem	>8	4.7
	Colistin	1	99.3
	Amikacin	>32	68.1
	Levofloxacin	>4	33.0
Asia/South Pacific (314)	Ceftazidime-avibactam	128	71.7
	Ceftazidime	>128	45.5
	Cefepime	>16	43.0
	Piperacillin-tazobactam	>128	30.6
	Doripenem	>4	9.2
	Meropenem	>8	0
	Imipenem	>8	5.7
	Colistin	1	99.0
	Amikacin	>32	78.7
	Levofloxacin	>4	39.5
Latin America (382)	Ceftazidime-avibactam	32	70.2
	Ceftazidime	>128	40.3
	Cefepime	>16	38.5
	Piperacillin-tazobactam	>128	29.1
	Doripenem	>4	7.6
	Meropenem	>8	0
	Imipenem	>8	5.0
	Colistin	1	99.0
	Amikacin	>32	56.8
	Levofloxacin	>4	30.1
Middle East/Africa (174)	Ceftazidime-avibactam	64	67.8
	Ceftazidime	128	48.3
	Cefepime	>16	42.5
	Piperacillin-tazobactam	>128	31.6
	Doripenem	>4	9.8
	Meropenem	>8	0
	Imipenem	>8	3.5
	Colistin	1	98.9
	Amikacin	>32	66.1
	Levofloxacin	>4	39.1

[a] Colistin was tested in the presence of a final concentration of 0.002% polysorbate 80.
[b] Values are based on CLSI breakpoints, except for ceftazidime-avibactam, for which FDA breakpoints were used.

zidime-avibactam on randomly selected clinical isolates of *P. aeruginosa* from Canada, China, the United States, and several European countries that reported MIC_{90} values of 4 to 8 μg/ml, with >90% of isolates susceptible (16–21, 23, 24). In all cases, the *in vitro* susceptibility of unselected isolates was greater to ceftazidime-avibactam than to meropenem, with the latter compound used as an "index" carbapenem (imipenem was used in one example where meropenem was not studied [17]).

In all regions, ceftazidime-avibactam was active against a proportion of ceftazidime-nonsusceptible (56.8 to 68.9% susceptible) and meropenem-nonsusceptible (67.8 to 74.2% susceptible) subsets of *P. aeruginosa* isolates. Together with amikacin and colistin, ceftazidime-avibactam retained the highest percentages of susceptibility against these subgroups of isolates. Similar percentages of ceftazidime-avibactam susceptibility were reported for such isolates collected in Canada, where ceftazidime-avibactam MIC values were ≤8 μg/ml for 66.1% of ceftazidime-resistant isolates and for 62.5% of meropenem-resistant isolates (16). Higher percentages of ceftazidime-avibactam susceptibility were reported among ceftazidime-nonsusceptible (80.9 to 82.1%) and meropenem-nonsusceptible (86.5 to 87.3%) isolates of *P. aeruginosa* from the United States (19, 21). These varied results highlight the importance of understanding, if possible, the makeup and underlying resistance mechanisms of selected subpopulations of isolates identified on the basis of phenotype or genotype, compared to a population of randomly selected clinical isolates. For example, there have been three studies of the activity of ceftazidime-avibactam against specific resistant subpopulations of *P. aeruginosa* (27–29). In these studies, it was convenient to summarize the subpopulations of isolates in terms of their 90th percentile MIC values. However, although these were abbreviated "MIC_{90}," it is important to distinguish them from the MIC_{90} obtained with a collection of randomly selected clinical isolates as is undertaken in surveillance (Table 1) (16–21, 23, 24), because the latter represents an estimate of the susceptibility of the contemporary clinical bacterial population (which the MIC_{90}s of the various selected resistant subpopulations do not do).

Carbapenem-nonsusceptible isolates were subjected to *bla* gene analysis. In agreement with other studies, very few acquired *bla* genes were found, 49.6% of which were bla_{VIM-2} (6, 7). Of the 563 ceftazidime-avibactam-resistant isolates identified in this study, 51.7% were metallo-β-lactamase positive and 13.0% carried genes for serine β-lactamases (KPC, SHV, VEB, PER, GES). Further work to understand the mechanism of resistance in such serine-β-lactamase-harboring isolates is needed. In 199 (35.3%) of ceftazidime-avibactam-resistant isolates, no acquired β-lactamase gene could be identified, implying either non-β-lactamase-mediated resistance mechanisms or the presence of *bla* genes not included in the molecular characterization protocol.

In conclusion, the *in vitro* behavior of ceftazidime-avibactam against *P. aeruginosa*, yielding percentages of susceptibility higher than those of other β-lactams tested, similar to those of amikacin, but lower than those of colistin, supports an ongoing clinical trial of this agent in patients with nosocomial pneumonia, including ventilated patients (41).

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