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Letter to the Editor

The *in vitro* activity of ceftazidime–avibactam against 417 Gram-negative bacilli collected in 2014 and 2015 at a teaching hospital in São Paulo, Brazil

Dear Editor:

Avibactam is a new non-β-lactam β-lactamase inhibitor that restores the *in vitro* activity of ceftazidime against isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* that harbor Ambler molecular class A, class C and some class D β-lactamases,[1] but not those harboring metallo-β-lactamases.[2] Ceftazidime–avibactam and comparator antibacterial agents were tested by reference broth microdilution against 417 non-repetitive Gram-negative bacilli (387 unselected, plus 30 selected bla_{KPC}-positive, meropenem–nonsusceptible, *Klebsiella pneumoniae*) collected prospectively from medical centers at Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil, in 2014 and 2015. Specimen sources were associated with bloodstream, respiratory, urinary, gynecological, intra-abdominal, wound, or skin and skin structure infections. Isolates defined as "non-selected" were collected without phenotypic pre-selection so that they represented those encountered clinically. Only one isolate per patient was included in the study. Minimum inhibitory concentrations (MICs), one per isolate, were determined by reference Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods[3] using frozen microtiter plates preloaded with antibiotic-containing growth medium. MICs of ceftazidime–avibactam were measured by varying the concentration of ceftazidime in twofold increments with avibactam at a fixed concentration of 4 mg/L.[3] Quality control (data not shown) was achieved according to CLSI guidelines[3] using American Type Culture Collection (ATCC) isolates, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853. The test results are summarized in Table 1.

Addition of avibactam at 4 mg/L decreased MICs of ceftazidime against unselected *Enterobacteriaceae*, especially *K. pneumoniae*, *Citrobacter freundii*, and *Enterobacter cloacae*, among which MIC_{90} values decreased from 128 to >128 mg/L to 0.5–4 mg/L. Among the unselected isolates of these three species 37–73% were susceptible to ceftazidime, whereas 100% were susceptible to ceftazidime–avibactam. The relatively large decreases in the MIC_{90} of ceftazidime caused by the addition of avibactam when testing clinical isolates of *C. freundii*, *Enterobacter aerogenes*, *E. cloacae*, *Morganella morganii*, *Providencia stuartii*, and *Serratia marcescens* likely resulted from the ceftazidime-nonsusceptible isolates of those species producing stably derepressed AmpC β-lactamases.[6] This is consistent with the observation that >90% of the isolates of those species remained susceptible to meropenem (Table 1). Of the 27 unselected isolates of *K. pneumoniae* tested, only 70.4% were susceptible to meropenem, and 37–41% were susceptible to the other β-lactam comparator agents tested, including the β-lactam/β-lactamase-inhibitor combination piperacillin-tazobactam. Susceptibility to levofloxacin was also low, at 40.7%, and almost 15% of the isolates lacked susceptibility to colistin. Of the 30 selected meropenem-nonsusceptible, bla_{KPC}-positive, isolates of *K. pneumoniae*: none was susceptible to ceftazidime alone, but 29 (96.7%) were susceptible to ceftazidime–avibactam *in vitro* (MIC_{90}, 4 mg/L). As expected, none of the β-lactam agents (except ceftazidime–avibactam) displayed appreciable activity against these isolates, and even susceptibility to colistin was compromised at 46.7% (Table 1). Susceptibility to amikacin was 90.0% (Table 1). This level of susceptibility of the bla_{KPC}-positive isolates to ceftazidime–avibactam is consistent with the results of the global surveillance studies of carbapenem-resistant *K. pneumoniae*.[2] In the case of *E. coli*, the range of MICs showed the avibactam effect, 0.12–64 mg/L for ceftazidime as opposed to 0.06–0.25 mg/L for ceftazidime–avibactam. Little to no avibactam effect was observed when testing ceftazidime–avibactam as opposed to ceftazidime against *Citrobacter koseri*, *Klebsiella oxytoca*, *Proteus vulgaris*, or *Providencia rettgeri*, as all isolates were already susceptible to ceftazidime.

Addition of avibactam decreased the MIC_{90} of ceftazidime against 25 unselected isolates of *P. aeruginosa* from >128 mg/L to 16 mg/L, resulting in 84% of isolates being interpreted as susceptible *in vitro* (Table 1), indicating a slightly lower level of susceptibility than has been found among isolates of this species in global surveillance studies. For example, in one recent global study, the MIC_{90} of ceftazidime–avibactam against 7062 isolates of *P. aeruginosa* was 8 mg/L, and 92% were susceptible.[7] Avibactam inhibits the AmpC β-lactamase of *P. aeruginosa* and restores susceptibility to ceftazidime in

Table 1 – *In vitro* activities of ceftazidime, ceftazidime–avibactam, and comparator antibiotics against *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* isolated from patients in São Paulo, Brazil, 2014–2015.

Organism (no. of isolates) and antibiotic	MIC (mg/L)			%S[a]	%R[a]
	MIC_{50}	MIC_{90}	Range		
Citrobacter freundii (25)					
Ceftazidime	0.5	>128	0.06 to >128	68.0	28.0
Ceftazidime–avibactam	0.25	0.5	0.06–1	100	0
Cefepime	≤0.06	8	≤0.06–32	80.0	8.0
Piperacillin/tazobactam	8	>128	2 to >128	72.0	20.0
Meropenem	0.25	0.5	0.12–4	96.0	4.0
Colistin	0.5	1	0.5–2	100	0
Amikacin	1	2	0.5–8	100	0
Levofloxacin	0.25	32	0.03 to >32	68.0	28.0
Citrobacter koseri (23)					
Ceftazidime	0.25	0.25	0.06–1	100	0
Ceftazidime–avibactam	0.12	0.25	0.06–0.5	100	0
Cefepime	≤0.06	0.12	≤0.06–0.5	100	0
Piperacillin/tazobactam	4	8	0.25–32	91.3	0
Meropenem	0.25	0.25	0.12–0.5	100	0
Colistin	0.5	0.5	0.25–0.5	100	0
Amikacin	1	2	0.12 to >128	95.7	4.3
Levofloxacin	0.03	0.25	≤0.015–1	100	0
Enterobacter aerogenes (25)					
Ceftazidime	0.25	64	0.06–64	72.0	28.0
Ceftazidime–avibactam	0.12	0.5	0.06–0.5	100	0
Cefepime	0.06	0.5	≤0.06–64	92.0	8.0
Piperacillin/tazobactam	4	128	2 to >128	68.0	24.0
Meropenem	0.25	1	0.25–4	96.0	4.0
Colistin	0.5	1	0.25–1	100	0
Amikacin	1	2	0.5–8	100	0
Levofloxacin	0.06	1	0.03–32	92.0	8.0
Enterobacter cloacae (26)					
Ceftazidime	0.5	128	0.12 to >128	73.1	26.9
Ceftazidime–avibactam	0.25	1	0.06–2	100	0
Cefepime	0.12	32	≤0.06 to >128	69.2	23.1
Piperacillin/tazobactam	4	128	1 to >128	76.9	11.5
Meropenem	0.25	0.5	0.12–1	100	0
Colistin	0.5	>32	0.5 to >32	76.9	23.1
Amikacin	1	4	0.5–16	100	0
Levofloxacin	0.06	8	0.03 to >32	88.5	11.5
Escherichia coli (27)					
Ceftazidime	0.25	1	0.12–64	96.3	3.7
Ceftazidime–avibactam	0.12	0.25	0.06–0.25	100	0
Cefepime	≤0.06	0.5	≤0.06–1	100	0
Piperacillin/tazobactam	4	16	2–64	92.6	0
Meropenem	0.25	1	0.12–2	96.3	0
Colistin	0.5	1	0.25–1	100	0
Amikacin	2	2	0.5–4	100	0
Levofloxacin	0.03	32	≤0.015–32	66.7	25.9
Klebsiella oxytoca (25)					
Ceftazidime	0.12	0.5	0.06–0.5	100	0
Ceftazidime–avibactam	0.12	0.5	0.03–0.5	100	0
Cefepime	≤0.06	0.12	≤0.06–0.5	100	0
Piperacillin/tazobactam	4	16	0.25 to >128	96.0	4.0
Meropenem	0.25	2	0.12–4	64.0	8.0
Colistin	0.5	1	0.25–4	96.0	4.0
Amikacin	1	8	0.5–16	100	0
Levofloxacin	0.06	1	≤0.015–8	96.0	4.0
Klebsiella pneumoniae (27)					
Ceftazidime	32	>128	0.06 to >128	37.0	63.0
Ceftazidime–avibactam	0.5	4	≤0.015–8	100	0
Cefepime	64	>128	≤0.06 to >128	37.0	55.6

Table 1 – (*Continued*)

Organism (no. of isolates) and antibiotic	MIC (mg/L)			%S[a]	%R[a]
	MIC_{50}	MIC_{90}	Range		
Piperacillin/tazobactam	128	>128	2 to >128	40.7	55.6
Meropenem	0.5	>32	0.12 to >32	70.4	29.6
Colistin	0.5	32	0.5 to >32	85.2	14.8
Amikacin	2	8	0.5–32	96.3	0
Levofloxacin	16	>32	0.06 to >32	40.7	59.3
Klebsiella pneumoniae (bla_{KPC}) (30)					
Ceftazidime	128	>128	8 to >128	0	93.3
Ceftazidime–avibactam	2	4	0.25–64	96.7	3.3
Cefepime	128	>128	2 to >128	3.3	96.7
Piperacillin/tazobactam	>128	>128	128 to >128	0	100
Meropenem	>32	>32	8 to >32	0	100
Colistin	8	>32	0.5 to >32	46.7	53.3
Amikacin	2	16	1 to >128	90.0	6.7
Levofloxacin	32	>32	0.06 to >32	6.7	93.3
Morganella morganii (25)					
Ceftazidime	0.5	8	0.06–128	80.0	12.0
Ceftazidime–avibactam	0.06	0.25	0.03–2	100	0
Cefepime	≤0.06	8	≤0.06 to >128	84.0	8.0
Piperacillin/tazobactam	1	8	0.25–128	92.0	4.0
Meropenem	1	1	0.5–1	100	0
Colistin	>32	>32	>32 to >32	0	100
Amikacin	4	8	1–8	100	0
Levofloxacin	1	>32	0.03 to >32	60.0	36.0
Proteus mirabilis (25)					
Ceftazidime	0.06	0.5	0.06–8	96.0	0
Ceftazidime–avibactam	0.06	0.06	0.03–0.12	100	0
Cefepime	0.12	0.5	≤0.06–32	92.0	4.0
Piperacillin/tazobactam	0.5	1	0.25–2	100	0
Meropenem	1	1	0.5–2	96.0	0
Colistin	>32	>32	>32 to >32	0	100
Amikacin	2	8	1–128	96.0	4.0
Levofloxacin	0.06	4	0.03–8	88.0	4.0
Proteus vulgaris (22)					
Ceftazidime	0.12	0.25	0.03–0.5	100	0
Ceftazidime–avibactam	0.06	0.12	0.03–0.25	100	0
Cefepime	0.12	0.5	≤0.06–2	100	0
Piperacillin/tazobactam	0.5	1	0.12–2	100	0
Meropenem	1	2	0.5–2	86.4	0
Colistin	>32	>32	>32 to >32	0	100
Amikacin	2	4	0.5–16	100	0
Levofloxacin	0.03	0.06	≤0.015–4	95.5%	0
Providencia rettgeri (14)					
Ceftazidime	0.12	0.5	0.06–0.5	100	0
Ceftazidime–avibactam	0.06	0.25	0.03–0.5	100	0
Cefepime	≤0.06	0.12	≤0.06–0.12	100	0
Piperacillin/tazobactam	1	4	0.5–4	100	0
Meropenem	0.5	0.5	0.12–0.5	100	0
Colistin	>32	>32	8 to >32	0	100
Amikacin	4	8	0.5–32	92.9	0
Levofloxacin	1	>32	0.12 to >32	57.1	42.9
Providencia stuartii (22)					
Ceftazidime	4	16	0.25 to >128	59.1	31.8
Ceftazidime–avibactam	0.5	1	0.12 to >128	95.5	4.5
Cefepime	>128	>128	≤0.06 to >128	22.7	72.7
Piperacillin/tazobactam	8	64	1–128	72.7	4.6
Meropenem	0.5	0.5	0.12–0.5	100	0
Colistin	>32	>32	>32 to >32	0	100
Amikacin	2	8	0.5–64	90.9	4.6
Levofloxacin	>32	>32	0.25 to >32	4.5	95.5

Table 1 – (*Continued*)

Organism (no. of isolates) and antibiotic	MIC (mg/L)			%S[a]	%R[a]
	MIC_{50}	MIC_{90}	Range		
Serratia marcescens (26)					
Ceftazidime	0.25	32	0.12 to >128	80.8	15.4
Ceftazidime–avibactam	0.25	1	0.06–32	96.2	3.8
Cefepime	0.25	>128	≤0.06 to >128	80.8	19.2
Piperacillin/tazobactam	4	>128	2 to >128	84.6	11.5
Meropenem	0.5	1	0.25–4	96.2	3.8
Colistin	>32	>32	>32 to >32	0	100
Amikacin	2	8	1–64	92.3	3.8
Levofloxacin	0.25	1	0.03–4	96.2	0
Pseudomonas aeruginosa (25)					
Ceftazidime	4	>128	0.5 to >128	60.0	40.0
Ceftazidime–avibactam	4	16	0.5–64	84.0	16.0
Cefepime	4	128	1 to >128	52.0	40.0
Piperacillin/tazobactam	16	>128	2 to >128	60.0	28.0
Meropenem	4	>32	1 to >32	32.0	32.0
Colistin	1	2	0.5–2	100	0
Amikacin	4	8	1–8	100	0
Levofloxacin	0.5	32	0.12–32	60.0	40.0
Acinetobacter baumannii (50)					
Ceftazidime	>128	>128	1 to >128	18.0	82.0
Ceftazidime–avibactam [b]	>128	>128	2 to >128	na[b]	na
Cefepime	64	>128	1 to >128	14.0	80.0
Piperacillin/tazobactam	>128	>128	≤0.06 to >128	14.0	82.0
Meropenem	>32	>32	2 to >32	16.0	84.0
Colistin	0.5	1	0.25–32	98.0	2.0
Amikacin	64	>128	1 to >128	30.0	56.0
Levofloxacin	16	>32	0.06 to >32	20.0	72.0

[a] %S, %R: percent of isolates interpreted as susceptible or resistant. MICs were interpreted according to CLSI criteria (breakpoints),[3] except for colistin against *Enterobacteriaceae* and ceftazidime–avibactam, for which CLSI criteria are not available. MICs of colistin against *Enterobacteriaceae* were interpreted by EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria.[4] MICs of ceftazidime–avibactam were interpreted according to criteria set by the United States Food and Drug Administration.[5] Accordingly, the susceptible and resistant criteria for the *Enterobacteriaceae* were (mg/L), respectively: ceftazidime, MIC ≤4 and ≥16; ceftazidime–avibactam, MIC ≤8 and ≥16; cefepime, MIC ≤2 and ≥16; piperacillin/tazobactam, MIC ≤ 16 and ≥128; meropenem, MIC ≤ 1 and ≥4; colistin, MIC ≤2 and >2; amikacin, MIC ≤16 and ≥64; and levofloxacin, MIC ≤2 and ≥8. Criteria for susceptible and resistant for *P. aeruginosa* were (mg/L), respectively: ceftazidime, MIC ≤8 and ≥32; ceftazidime–avibactam, MIC ≤8 and ≥16; cefepime, MIC ≤8 and ≥32; piperacillin/tazobactam, MIC ≤16 and ≥128; meropenem, MIC ≤2 and ≥8; colistin, MIC ≤2 and ≥8; amikacin, MIC ≤16 and ≥64; and levofloxacin, MIC ≤2 and ≥8. The criteria used to interpret MIC values against *A. baumannii* were identical to those used for *P. aeruginosa* except for ceftazidime–avibactam, which lacks a breakpoint because the drug label does not include *Acinetobacter* spp., and colistin, the criterion of resistance to which was MIC ≥4 mg/L.

[b] na: not applicable because *A. baumanni* is not an indicated species for ceftazidime–avibactam.[5]

isolates in which the mechanism of resistance is that of stable derepression of that enzyme.[8] However, multidrug-resistant *P. aeruginosa* clone ST277 that frequently harbors metallo-β-lactamase SPM-1 is disseminated widely in Brazil[8] and avibactam does not inhibit metallo-β-lactamases, which might explain the 16% resistance to ceftazidime–avibactam found here (Table 1). Nevertheless, the percent of the *P. aeruginosa* isolates in the present study that were susceptible to ceftazidime–avibactam was higher than the percent that were susceptible to the other β-lactam agents tested, including meropenem (32% susceptible) and piperacillin/tazobactam (60% susceptible) (Table 1).

The MIC_{90} of ceftazidime against 50 isolates of *Acinetobacter baumannii* was >128 mg/L whether avibactam was present or not, which is consistent with this species not being listed on the drug label.[5]

In conclusion, the *in vitro* antibacterial activity of ceftazidime–avibactam against bacteria isolated from patients in Brazil was consistent with results from other surveillance studies except that the percent susceptibility of the sample of clinical isolates of *P. aeruginosa* at 84% (Table 1) was somewhat lower than the proportions observed elsewhere,[7] possibly related to the dissemination of the metallo-β-lactamase, SPM-1, in Brazil.[8] The *in vitro* activity of ceftazidime–avibactam described in the present work is consistent with its recently-reported efficacy in phase 3 clinical studies.[9]

Conflicts of interest

WW Nichols and R Testa were employees of AstraZeneca at the time of the study and WWN is an AstraZeneca shareholder. Pfizer acquired the AstraZeneca product, ceftazidime-avibactam, in December 2016. The Hospital das Clinicas da Universidade de São Paulo Group declare no conflicts of interest.

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Flávia Rossi [a], Ana P. Cury [a], Maria R.G. Franco [a], Raymond Testa [b], Wright W. Nichols [b,*,◊]

[a] *Universidade de São Paulo, Hospital das Clínicas da Faculdade de Medicina, Seção de Microbiologia, Divisão de Laboratório Central LIM03, São Paulo, SP, Brazil*

[b] *Formerly of AstraZeneca Pharmaceuticals LP, Waltham, MA, USA*

[*] *Corresponding author.*
E-mail address: wrightnichols1@gmail.com (W.W. Nichols).
[◊] Current address: Cambridge, MA, USA.

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