In Vitro Activity of Ceftazidime-Avibactam Combination in In Vitro Checkerboard Assays

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To evaluate the in vitro effects of the combination of ceftazidime and avibactam on the MICs of both compounds, checkerboard assays were performed for 81 clinical strains, including 55 Enterobacteriaceae strains (32 Klebsiella pneumoniae, 19 Escherichia coli, 1 Citrobacter freundii, and 3 Enterobacter cloacae) and 26 strains of Pseudomonas aeruginosa, all with known resistance mechanisms such as extended-spectrum β-lactamases (ESBLs) and carbapenemases, phenotypically or molecularly determined. Phenotypically ceftazidime-resistant strains (n = 69) were analyzed in more detail. For the Enterobacteriaceae strains, a concentration-dependent effect of avibactam was found for most strains with a maximum effect of avibactam at a concentration of 4 mg/liter, which decreased all ceftazidime MICs to ≤4 mg/liter. Avibactam alone also showed antibacterial activity (the MIC50 and MIC90 being 8 and 16 mg/liter, respectively). For the ceftazidime-resistant P. aeruginosa strains, considerable inhibition of β-lactamases by avibactam was acquired at a concentration of 4 mg/liter, which decreased all ceftazidime MICs except one to ≤8 mg/liter (the CLSI and EUCAST susceptible breakpoint). Increasing the concentration of avibactam further decreased the MICs, resulting in a maximum effect for most strains at 8 to 16 mg/liter. In summary, for most strains, the tested addition of avibactam of 4 mg/liter restored the antibacterial activity of ceftazidime to a level comparable to that of wild-type strains, indicating full inhibition, and strains became susceptible according to the EUCAST and CLSI criteria. Based on these in vitro data, avibactam is a promising inhibitor of different β-lactamases, including ESBLs and carbapenemases.

Antibiotic resistance is a worldwide problem. Until the past decade, the development of new classes of antibiotics was an important weapon against development of resistance. However, strains that carry extended-spectrum β-lactamases and/or carbapenemases have emerged (1, 2). In some countries, the resistance levels to these drugs are now >50% (3). One approach for overcoming this mechanism of resistance is by inhibition of these enzymes. This approach has been taken in the past against β-lactamase-carrying strains using drug combinations consisting of a β-lactam agent and a β-lactamase inhibitor (4–11), such as piperacillin-tazobactam and amoxicillin-clavulanic acid. These have been and still are among the most successful antimicrobials available.

Among several new β-lactamase inhibitors and combinations with β-lactams that are currently being developed is avibactam (12, 13). This compound is active against Ambler class A extended-spectrum β-lactamases (ESBLs) (e.g., TEM, SHV, and CTX-M types), KPC class A enzymes, class C (AmpC), and some class D enzymes. Studies in vitro (14–24) have shown that the MICs of ceftazidime for many resistant strains were markedly reduced in the presence of avibactam and thereby became susceptible.

An important step in the development of a combination β-lactam–β-lactamase inhibitor is to determine the concentration-effect relationship and the concentration at which maximal in vitro inhibition is achieved. These relationships can subsequently be used to determine the optimal conditions to correlate the in vitro testing with the efficacy of the combination. Therefore, we employed a checkerboard assay (25) to evaluate the in vitro effects of ceftazidime combined with avibactam over a range of concentrations for a selection of clinical Gram-negative isolates, including Enterobacteriaceae and Pseudomonas aeruginosa.

MATERIALS AND METHODS

Antibiotics. Ceftazidime (lot no. G263848; potency 77.0%) and avibactam (lot no. AFCH005151; potency 91.7%) were provided by AstraZeneca Pharmaceuticals LP (Waltham, MA, USA). The drugs were reconstituted in sterile water to a stock solution of 5,120 mg/liter, and further solutions were prepared in Mueller-Hinton broth (Difco; Bruenschwig Chemie, Amsterdam, The Netherlands).

Bacterial strains. Originally, 81 ceftazidime-resistant strains were obtained, all from clinical samples from a wide variety of infections, one strain per patient. The strains were chosen because they represent different β-lactamase-mediated mechanisms of resistance and thus did not quantitatively represent the presence of such resistance mechanisms in the general population, i.e., unselected clinical isolates. Of the isolates, 69 were retained after susceptibility testing, because they were phenotypically ceftazidime resistant or intermediate according to the MIC and EUCAST interpretive criteria. A list describing the strains is provided in Table 1. Included in this selection were 51 Enterobacteriaceae (18 Escherichia coli, 29 Klebsiella pneumoniae, 3 Enterobacter cloacae, and 1 Citrobacter freundii) and 18 P. aeruginosa isolates.

Susceptibility testing. The MICs of ceftazidime and avibactam were determined by microdilution according to the ISO guidelines (26). The drugs were reconstituted in sterile water to a stock solution of 5,120 mg/liter (the CLSI and EUCAST susceptible breakpoint). Increasing the concentration of avibactam further decreased the MICs, resulting in a maximum effect for most strains at 8 to 16 mg/liter. In summary, for most strains, the tested addition of avibactam of 4 mg/liter restored the antibacterial activity of ceftazidime to a level comparable to that of wild-type strains, indicating full inhibition, and strains became susceptible according to the EUCAST and CLSI criteria. Based on these in vitro data, avibactam is a promising inhibitor of different β-lactamases, including ESBLs and carbapenemases.
liter, and further solutions were prepared in Mueller-Hinton broth (Difco; Brunschwig Chemie). Checkboards were set up with 2-fold dilutions of ceftazidime (0.032 to 256 mg/liter) and avibactam (0.016 to 16 mg/liter). Freshly prepared trays were stored at 80°C until use. Every tray contained a negative control and a growth control. Each set of MIC determinations included three control strains: E. coli ATCC 25922, P. aeruginosa ATCC 27853, and K. pneumoniae ATCC 700603. Three to 5 μl of 1.5 × 10² CFU/ml bacterial suspension was added in each well with an inoculator (INOC 2001; Bitel Mechatronics B.V., INOC B.V., Zevenbergen, The Netherlands). Plates were read after 18 to 20 h of incubation at 35°C in a closed plastic box using a mirrored surface. The MIC was recorded as the lowest concentration of the agent that completely inhibited visible growth. All experiments were performed in duplicate.

**Analysis.** The susceptibility of the combination was interpreted following the EUCAST criteria for ceftazidime (27) (Enterobacteriaceae: susceptible, ≤1 mg/liter; resistant, >4 mg/liter; P. aeruginosa: susceptible, ≤8 mg/liter; resistant, >8 mg/liter). Results are expressed as the MIC₅₀ and MIC₉₀ of ceftazidime and the concentrations of avibactam required to reduce the MIC of ceftazidime (Prism, version 6, 2013; GraphPad Software, San Diego, CA).

**RESULTS**

Avibactam alone did not have a significant inhibitory effect on P. aeruginosa nor on C. freundii isolates, whereas it did have an inhibitory effect on other Enterobacteriaceae strains, with a MIC of 8 mg/liter (the majority of E. coli and K. pneumoniae strains) or 16 mg/liter (1 of the 3 E. cloaca strains) for avibactam in the absence of ceftazidime (data not shown).

**Figures 1 and 2** show the effects of increasing concentrations of avibactam on the MIC of ceftazidime as cumulative inhibition for P. aeruginosa isolates and Enterobacteriaceae strains, respectively. The cumulative inhibition plots are shifted to the left for increasing concentrations of avibactam, indicating a clear effect: the higher the concentration of avibactam present, the lower the resulting MIC for ceftazidime.

**Figures 2** shows the MIC₅₀ and MIC₉₀ values of ceftazidime at various concentrations of avibactam and provides an indication of the concentration of avibactam required to reduce the MICs for ceftazidime for Enterobacteriaceae and P. aeruginosa strains. A reasonable shift of MICs was already attained with the addition of only 0.016 mg/liter avibactam. The impact of avibactam on the Enterobacteriaceae strains was more pronounced than for the P. aeruginosa isolates. A maximum concentration of avibactam of 4 mg/liter resulted in susceptibility for Enterobacteriaceae strains, whereas for P. aeruginosa 8 to 16 mg/liter avibactam was required to reach susceptible levels for all strains.

**Figure 3** shows the maximum decrease in ceftazidime MICs in number of 2-fold dilutions. Addition of avibactam to ceftazidime reduced the MIC of ceftazidime up to 7 to 9 2-fold dilutions for the majority of the Enterobacteriaceae strains, and the maximum effect was reached at an avibactam concentration of 4 mg/liter. For the P. aeruginosa strains, the maximum effect was less pronounced: 5 to 6 doubling dilutions and higher concentrations of avibactam, up to 8 to 16 mg/liter, were required. One P. aeruginosa

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**TABLE 1 Resistance specifications of the 69 ceftazidime-resistant clinical isolates used in the checkerboard assay**

<table>
<thead>
<tr>
<th>No. of strains</th>
<th>Genus and species</th>
<th>Resistance specifications¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Ceftazidime resistant, carbapenem resistant, AmpCcon, AmpCind, AmpC derepressed, AmpC inducible</td>
</tr>
<tr>
<td>29</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>OmpK35, OmpK36, KPC-2 and -3, CTX-M-15, gr. 9, 39, TEM-1 and -84, SHV-1, -2, -5, -11, -12, and -33, OXA-1, -2, and -48, LEN, GES-1, ESBL, possible carbapenemases, AmpC con, AmpC derepressed; AmpC inducible</td>
</tr>
<tr>
<td>18</td>
<td><em>Escherichia coli</em></td>
<td>CTX-M-1, -2, -3, and -15, TEM-1 and -84, SHV-1 and -12, OXA-1, ESBL</td>
</tr>
<tr>
<td>3</td>
<td><em>Citrobacter freundii</em></td>
<td>CTX-M-9, TEM-1, SHV-12, OXA-1</td>
</tr>
<tr>
<td>1</td>
<td><em>Enterobacter cloacae</em></td>
<td>KPC-2</td>
</tr>
</tbody>
</table>

¹ AmpCcon, AmpC derepressed; AmpCind, AmpC inducible.

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**FIG 1** Cumulative % inhibition of 18 P. aeruginosa isolates by ceftazidime for increasing concentrations (symbols below the figure, mg/liter) of avibactam. The % inhibition of growth of P. aeruginosa is presented on the y axis with the MIC for ceftazidime on the x axis as related to the added concentrations of avibactam in differently colored lines.
isolate did not show any change in the MIC of ceftazidime in combination with the highest concentration of avibactam added.

Table 3 displays target MICs of ceftazidime for Enterobacteriaceae and P. aeruginosa isolates and the required concentrations of avibactam expressed as the 50th and 90th percentiles. The median and 90th percentile concentrations of avibactam required to reduce the MIC of ceftazidime to 4 mg/liter were 4 and >16 mg/liter for P. aeruginosa strains, 0.016 and 0.063 mg/liter for E. coli strains, and 0.250 and 1 mg/liter for K. pneumoniae strains respectively. The median and 90th percentile concentrations of avibactam required to reduce the MIC of ceftazidime to 1 mg/liter were 32 and ≥64 mg/liter (i.e., the measurable MIC<sub>90</sub> was not attained) for P. aeruginosa strains, 0.031 and 2 mg/liter for E. coli strains, and 2 and 8 mg/liter for K. pneumoniae strains, respectively.

At an avibactam concentration of 1 mg/liter, the MIC<sub>50</sub> of ceftazidime against the isolates of P. aeruginosa was at or below the ceftazidime resistance breakpoint of 8 mg/liter (as shown in Table 2). The MIC<sub>90</sub> however, was much higher (32 mg/liter). At a concentration of avibactam of 4 mg/liter (as currently used for <i>in vitro</i> broth dilution susceptibility testing) (28), the MIC<sub>90</sub> for the ceftazidime-avibactam combination was 16 mg/liter, one dilution higher than both the EUCAST and CLSI breakpoints of ceftazidime for P. aeruginosa. The median and 90th percentile concentrations of avibactam required to reduce the MIC of ceftazidime to 1 mg/liter were 32 and 64 mg/liter (i.e., the measurable MIC<sub>90</sub> was not attained) for P. aeruginosa isolates, 0.031 and 2 mg/liter for E. coli strains, and 2 and 8 mg/liter for K. pneumoniae strains respectively.

Likewise the MIC<sub>50</sub> and MIC<sub>90</sub> of ceftazidime-avibactam for Enterobacteriaceae strains decreased with increasing concentrations of avibactam. Of note, avibactam showed some moderate

![FIG 2](https://example.com/fig2.png) Cumulative % inhibition of 51 Enterobacteriaceae isolates by ceftazidime for increasing concentrations (symbols below the figure, mg/liter) avibactam. The % inhibition of growth of Enterobacteriaceae is presented on the y axis with the MIC for ceftazidime on the x axis as related to the added concentration of avibactam in differently colored lines.

<table>
<thead>
<tr>
<th>Avibactam concn (mg/liter)</th>
<th>P. aeruginosa (n = 18)</th>
<th>All Enterobacteriaceae (n = 51)</th>
<th>E. coli (n = 18)</th>
<th>K. pneumoniae (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/liter)</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt; (mg/liter)</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/liter)</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt; (mg/liter)</td>
</tr>
<tr>
<td>0</td>
<td>32</td>
<td>128</td>
<td>32</td>
<td>≥512</td>
</tr>
<tr>
<td>0.016</td>
<td>32</td>
<td>128</td>
<td>8</td>
<td>128</td>
</tr>
<tr>
<td>0.031</td>
<td>32</td>
<td>128</td>
<td>4</td>
<td>64</td>
</tr>
<tr>
<td>0.063</td>
<td>32</td>
<td>128</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>0.125</td>
<td>32</td>
<td>128</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>0.25</td>
<td>32</td>
<td>128</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>0.5</td>
<td>16</td>
<td>64</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>32</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>16</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>16</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>8</td>
<td>≤0.031</td>
<td>0.25</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>8</td>
<td>≤0.031</td>
<td>≤0.031</td>
</tr>
<tr>
<td>32</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
antibacterial activity without ceftazidime (range, 8 to >16 mg/liter; MIC$_{50}$ and MIC$_{90}$ of avibactam, respectively, 8 and 16 mg/liter). This resulted in a very low MIC$_{50}$ and MIC$_{90}$ of the ceftazidime-avibactam combination for Enterobacteriaceae strains. For all the Enterobacteriaceae strains tested, at a concentration of 1 mg/liter of avibactam, the MIC$_{90}$ of the combination was at or below the ceftazidime-susceptible breakpoint of 4 mg/liter. The effect of avibactam was species dependent, the MICs of the combination against E. coli isolates being reduced to a MIC$_{90}$ of 2 mg/liter when the concentration of avibactam was 0.063 mg/liter, whereas 1 mg/liter avibactam was required to bring the MIC$_{90}$ of the combination against K. pneumoniae isolates to 4 mg/liter.

**DISCUSSION**

In this study, it was shown that the addition of avibactam renders ESBL-producing strains susceptible to ceftazidime, but that the concentration of avibactam required is species and strain dependent. Although the efficacy of the combination of avibactam and ceftazidime in vitro was described in earlier studies for some concentrations of avibactam (14, 16, 18, 21, 23), here we present the results of complete checkerboard experiments.

It should be noted that the isolates of Enterobacteriaceae and P. aeruginosa studied in the present work were selected for further investigation due to ceftazidime resistance, i.e., the ceftazidime or ceftazidime-avibactam MIC distributions of the isolates studied are not representative of the normal MIC distribution observed in a routine clinical laboratory. The median and 90th percentile tables and the cumulative frequency plots are ways of displaying the collected properties of the sample of isolates chosen. They are not in any way estimates of the MIC distribution statistics of any current population of clinical isolates. The value of this collection is as a challenge set of bacteria that can be used to study the concentration-effect profile of avibactam when combined with ceftazidime against β-lactamase-producing isolates. These baseline results were important for ensuing pharmacokinetic/pharmacodynamic (PK/PD) studies (e.g., reference 29).

The results of the checkerboard experiments were analyzed in different ways. First, the maximum effective concentration of avibactam was determined for the different microorganisms and species. From these data, it can be concluded that the activity of avibactam in combination with ceftazidime is more pronounced for Enterobacteriaceae than for P. aeruginosa strains. The possible explanations include the existence of more and different resistance mechanisms present in Pseudomonas species compared to the Enterobacteriaceae, such as resistance due to changes in porins or efflux pumps (30, 31).

Except for one strain of P. aeruginosa, avibactam restored the antibacterial activity of ceftazidime to a level comparable to that of wild-type strains (even though a concentration of 32 mg/liter avibactam was required to reach this for strain 11), which renders them susceptible according to both the CLSI and the EUCAST criteria. This is consistent with what was observed for a collection of unselected clinical isolates of P. aeruginosa where the addition of avibactam at 4 mg/liter restored the antibacterial activity of ceftazidime (32) and reverted the frequency distribution of the MICs of ceftazidime to one resembling the EUCAST “wild-type” distribution (33).

Second, the optimum inhibitory combinations of ceftazidime and avibactam in vitro were derived from the data. Ideally, the concentration of avibactam to use is such that the majority of strains are inhibited by ceftazidime-avibactam and, on the other hand, that distinguishes between strains that harbor enzymes that are susceptible or resistant to avibactam inhibition at the putative breakpoint level of ceftazidime-avibactam. This is not necessarily the same as the breakpoint for ceftazidime owing to considerations of dosing, human population pharmacokinetics, PK/PD indices and magnitudes, MIC frequency distributions in surveil-

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**TABLE 3 Concentration of avibactam required to reach a given MIC of ceftazidime for the median and 90th percentile of strains**

<table>
<thead>
<tr>
<th>Ceftazidime target MIC (mg/liter)</th>
<th>MICs of avibactam (50th/90th percentiles [mg/liter]) for:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. aeruginosa (n = 18)</td>
<td>E. coli (n = 18)</td>
</tr>
<tr>
<td>1</td>
<td>32/≥64</td>
<td>1/4</td>
</tr>
<tr>
<td>4</td>
<td>4/16</td>
<td>0.063/1</td>
</tr>
<tr>
<td>8</td>
<td>1/8</td>
<td>0.031/0.25</td>
</tr>
</tbody>
</table>

**FIG 3** Distribution of the maximum effect of ceftazidime-avibactam combinations over the tested concentration range expressed as a 2-fold dilution of the MIC decrease of ceftazidime. The maximum MIC-lowering effect of the addition of avibactam to ceftazidime (compared to the MIC of ceftazidime alone) for % strains of both Enterobacteriaceae and P. aeruginosa is presented on a 2-log transformed x axis.
lance studies, activity against key multidrug-resistant strains, and clinical data.

At an avibactam concentration of 1 mg/liter, the MIC_{90} of ceftazidime against the isolates of *P. aeruginosa* was at or below the ceftazidime resistance breakpoint of 8 mg/liter (note that this breakpoint is associated with the 2-g, every 8 h [q8h] dose of ceftazidime, whereas the ceftazidime breakpoint for *Enterobacteriaceae* of 4 mg/liter was set based on the 1-g, q8h dose [EUCAST ceftazidime breakpoint rationale document (34)]. The MIC_{90} of ceftazidime at the avibactam concentration of 1 mg/liter was much higher (32 mg/liter).

In the presence of a fixed amount of 4 mg/liter avibactam, 92% of all of the bacterial strains tested displayed a MIC to the combination of ≤4 mg/liter. However, a number of strains of *P. aeruginosa* required significantly higher concentrations of avibactam in the range of 8 to 32 mg/liter (*in vitro*) to achieve the ceftazidime-susceptible breakpoint. The current dosing (2,000 mg ceftazidime and 500 mg avibactam q8h, 2-h infusion per dose [35]) will most likely not result in concentrations high enough or long enough to inhibit these specific strains *in vivo*.

It should be noted that the *P. aeruginosa* isolates selected for this study represented isolates from the high end of the MIC distribution of current clinical isolates (14, 32) in order to identify strains suitable for PK/PD analyses in murine infection studies (e.g., see reference 29). The concentration of avibactam used in susceptibility testing cannot be related directly to the fluctuating avibactam concentrations *in vivo*. Rather, the *in vitro* test is designed so that a constant concentration of avibactam inhibits β-lactamasms as much as is practicable in order to reveal the intrinsic susceptibility to ceftazidime which is diluted in 2-fold increments, read as the “MIC.” *In vivo*, the question becomes what PK time course of concentrations of avibactam will inhibit β-lactamases and support the underlying intrinsic PK/PD of avibactam? (29, 36, 37). That is outside the scope of the present work, but suffice it to say that it need not be related exactly to the 4 mg/liter avibactam adopted as the fixed susceptibility test concentration (28).

The resulting data provide a basis for determining the optimal avibactam concentration in routine susceptibility testing in microdilution assays. The data indicate that a concentration of avibactam of 4 mg/liter will give the best result while ensuring that the concentration remains below the point at which avibactam begins to show some antibacterial activity alone in the case of *Enterobacteriaceae* strains (Table 1), which might potentially yield *in vitro* test results of false susceptibility (i.e., very major errors). This is consistent with the currently adopted standard method set by the CLSI (28, 38).

The MIC-lowering effect was far more pronounced for the *Enterobacteriaceae* strains. For most strains, 1 mg/liter of avibactam was enough to reach the maximum β-lactamase inhibition, and the β-lactamases of virtually all strains were inhibited at 4 mg/liter. The β-lactamases expressed in *E. coli* isolates in particular were very susceptible to avibactam with concentrations as low as 0.063 mg/liter reinstating susceptibility to ceftazidime, whereas for the *K. pneumoniae* and other *Enterobacteriaceae* isolates, 1 mg/liter was necessary. The stepwise increase in ceftazidime susceptibility with increasing avibactam concentration leads to the hypothesis that multiple steps interact in the restoration of ceftazidime activity by avibactam and that each step contributes to a different extent in different strains. For *K. pneumoniae*, avibactam at 4 mg/liter brought MIC values of ceftazidime against all of the challenge isolates in this selected sample to ≤4 mg/liter (Table 1) and of 90% to ≤2 mg/liter (Table 2). In the attempt to relate this observation to the clinical standards of the MIC interpretive criteria, it is relevant to note that the PK/PD target attainment based cutoff MIC for the 1-g, q8h dose of ceftazidime is 4 mg/liter (28, 34, 38). The current EUCAST ceftazidime-susceptible breakpoint of ≤1 mg/liter was lowered from the PK/PD cutoff of 4 mg/liter in order to avoid categorization of ESBL producers inhibited by 2 or 4 mg/liter as susceptible (34). However, the combination of ceftazidime with avibactam is designed to overcome the hydrolysis by ESBLs, and, thus, the PK/PD target attainment based on the 1-g, q8h ceftazidime dose might support a susceptible breakpoint of ≤4 mg/liter. Indeed, this is currently the CLSI MIC-based interpretive criterion of ceftazidime susceptible (28). In the case of ceftazidime-avibactam, the dose is 2 g ceftazidime (plus 0.5 g avibactam), q8h, and the infusion time is 120 min rather than the standard 30 min used for ceftazidime, so the PK/PD cutoff is proportionately higher (35, 39). It should be noted that the PK/PD cutoff for the 2-g, 8-h dose of ceftazidime brought MIC values against the challenge isolates reported here to any putative susceptibility interpretive criterion, the appropriate value to use ultimately will be the PK/PD cutoff of the q8h 120-min infusion dose of 2 g ceftazidime combined with 0.5 g avibactam, the definitive analysis of which has yet to be performed, because phase 3 clinical trials are in progress (but see references 35 and 39 for pre-phase 3 models and analyses).

The MICs for ceftazidime were also lowered for phenotypically ceftazidime-susceptible strains (data not shown). This can possibly be explained by a baseline level of β-lactamase production that elevates the baseline MIC of ceftazidime, in which case avibactam potentially removes that baseline activity. Hitherto, the mechanism of synergy between avibactam and ceftazidime had not been investigated.

Since it might be possible that there was a specific trend in the results, depending on the selected microorganisms or on the resistance mechanism these microorganisms possessed, we specifically looked at whether this was the case. No clear differences related to the type of ESBL present or to the presence of one or more ESBLs were visible, although there are biochemical kinetic differences in the inhibition of different β-lactamases *in vitro* (12). Another factor that might affect the ability of avibactam to lower the MICs of ceftazidime is that the number of copies per cell may differ, requiring more or less avibactam.

Similar to earlier observations, we did not find any intrinsic antipseudomonal activity of avibactam alone (MICs of ≥16 or 64 mg/liter) (18, 24), and the primary use of avibactam is the inhibition of β-lactamases, including multidrug-resistant (MDR) strains. Ceftazidime activity was restored from 40% to 96% in 25 MDR *P. aeruginosa* isolates (18), where MDR was defined as resistance to at least one antimicrobial agent from three or more different classes. The molecular resistance mechanisms were not mentioned in this study. Similarly, Levasseur et al. (14) showed an increase in the ceftazidime susceptibility from 65% to 94% with the addition of 4 mg/liter avibactam in 126 consecutive clinical *P. aeruginosa* isolates. The activity against AmpC−mediated resistance was shown by Mushtaq and colleagues when they demon-
strated that avibactam reversed the AmpC-mediated ceftazidime resistance in *P. aeruginosa* isolates, by reducing the MICs for fully derepressed mutants and isolates to ≤8 mg/liter (21). The 6 *Pseudomonas* isolates carrying AmpC in the present study also turned susceptible with addition of avibactam.

For *Enterobacteriaceae*, avibactam has shown significant activity against class A, C, and some D β-lactamases but not to metallo-β-lactamases (24). These include KPC-producing strains (23), including ESBLs and KPC-type carbapenemases, and might be an alternative treatment option for infections caused by ESBL-harboring strains. The checkerboard approach that we took indicates that there is a concentration-dependent effect of avibactam. Whereas the 4 mg/liter used in susceptibility testing brought the MICs to ≤2 mg/liter (the CLSI breakpoint and the EUCAST PK/PD cutoff for the 1-g, q8h dose) and against the majority of the *P. aeruginosa* isolates in the present study to ≤8 mg/liter (the CLSI and EUCAST breakpoints), the concentration dependence also raises the hypothesis that higher exposures of avibactam, if proven safe, might ultimately lead to more isolates being susceptible in vivo, particularly among multidrug-resistant *P. aeruginosa* strains.

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