Avibactam confers susceptibility to a large proportion of ceftazidime-resistant

*Pseudomonas aeruginosa* isolates recovered from cystic fibrosis patients

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Sir,

*Pseudomonas aeruginosa* is the predominant bacterial pathogen in cystic fibrosis (CF) patients and associated with decline in pulmonary function.\(^1\) Due to the chronic persistent nature of infections, CF patients receive frequent antibiotic courses for eradication of potential pathogens, treatment of acute infective exacerbations, and as chronic suppressive therapy. Consequently, resistance to antipseudomonal \(\beta\)-lactams is common in the strains collected from CF patients,\(^2,3\) narrowing therapeutic options. Clinicians are therefore forced to use aminoglycosides or polymyxins, increasing the risk of adverse effects.\(^4,5\) Therefore, optimizing the activity of \(\beta\)-lactams may help to alleviate this burden. Ceftazidimen is a well-established cephalosporin (on the World Health Organization's List of Essential Medicines) with excellent safety profile and antibacterial spectrum that includes *P. aeruginosa*. However, ceftazidime is degraded by many \(\beta\)-lactamases including extended-spectrum \(\beta\)-lactamases (ESBLs; Ambler class A and D), cephalosporinases (Ambler class C), and carbapenemases. Avibactam (formerly NXL-104) is a novel non-\(\beta\)-lactam, broad-spectrum \(\beta\)-lactamase inhibitor, with promising inhibitory activity towards Ambler class A (including ESBLs and *Klebsiella pneumoniae* carbapenemases [KPC]), C and D \(\beta\)-lactamases.\(^6\) Combined with ceftazidime, it is currently in phase III clinical trials for the treatment of complicated intra-abdominal infections, urinary tract infections and healthcare-associated pneumonia (http://clinicaltrials.gov, identifiers NCT01499290; NCT01500239; NCT01726023; NCT01644643; NCT01595438; NCT01808092). In *P. aeruginosa* from non-CF patients, avibactam has been shown to reverse ceftazidime resistance, bringing MICs to values lower than the EUCAST and CLSI breakpoints.\(^7,8\) However, very little is known about the effect of avibactam on ceftazidime activity in *P. aeruginosa* isolated from CF patients.\(^9\) We therefore assembled a collection of 334 non-duplicate *P. aeruginosa* isolates from 156 patients with a clinically-confirmed diagnostic of CF equally distributed between 4 European countries with a predominance of recent isolates (Belgium [2010], France [1996-2012], Germany [2012] and
United Kingdom [2006-2009]) and used them to assess the activity of ceftazidime alone or combined with avibactam. MICs were determined by microdilution in cation-adjusted Muller Hinton broth following the CLSI methodology for ceftazidime alone (procured as Glazidim®, the commercial product registered in Belgium for parenteral use; potency, 88.2%; Glaxo-SmithKline; Genval, Belgium) and combined with 4 mg/L avibactam (NXL-104, potency 91.7%, batch no. AFCH005151; AstraZeneca Pharmaceuticals, Waltham, MA, USA). P. aeruginosa ATCC 27853 (fully susceptible) and K. pneumoniae ATCC 700603 (resistant to ceftazidime by the production of SHV-18 β-lactamase) were used as quality controls. Correlations between MICs of ceftazidime and ceftazidime/avibactam against individual strains were assessed using quantile density contour analysis (JMP® versions 10.0.2, SAS Institute Inc, Cary, NC). Panel A of the figure shows that isolates in this collection had a large MIC90 for ceftazidime (512 mg/L) with only 36% being clinically susceptible (MIC ≤ 8 mg/L) according to EUCAST or CLSI interpretive criteria. When combined with avibactam, the proportion of susceptible strains increased to 76% and the MIC90 decreased to 64 mg/L. Panel B of the figure shows the fold reduction in MIC observed in the presence of avibactam for these isolates classified according to the MIC of ceftazidime. While the mean reduction in MIC observed for the whole collection was 2.6 dilutions, the amplitude of the effect was clearly dependent on the initial ceftazidime MIC. Thus, when combined with avibactam, the MIC of ceftazidime decreased by 0.6 dilutions for each doubling of ceftazidime MIC in the 1 to 128 mg/L range (0.6 is the slope value of a linear regression relating the log₂ MIC of the combination to the log₂ MIC of ceftazidime in that range; R² 0.965), which would decrease the MIC to 8-16 mg/L, irrespective of the ceftazidime MIC in that range of concentrations. For more resistant strains, the amplitude of the avibactam effect plateaued at a reduction of about 4 dilutions in MIC for strains for which the ceftazidime MIC was about 256 mg/L and decreased to a reduction of 3 dilutions for isolates for which the MICs were still higher. This shift in MIC is illustrated for individual strains in panel C of the figure, which
shows the correlation between MICs of individual isolates for ceftazidime alone and ceftazidime combined with avibactam. Susceptibility to ceftazidime was restored in 40% of the strains, with avibactam proving more effective for strains harbouring an MIC < 256 mg/L. In accordance with the conclusion drawn from panel C of the figure, the ceftazidime MIC was now only 4-8 mg/L for most of the affected strains, a value that is below the EUCAST and CLSI susceptibility breakpoints, extending to CF *P. aeruginosa* isolates the conclusions obtained for pseudomonal isolates from other origin\(^7\) and for other Gram-negative bacteria.\(^8\)

Taken together, these data highlights the potential utility of combining ceftazidime with avibactam for the treatment of *P. aeruginosa* infections, including in clinical situations where resistance rates are high. It also shows that a concentration of 4 mg/L is sufficient to bring to the susceptible range those *P. aeruginosa* strains with a ceftazidime MIC ≤ 256 mg/L.
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References


**Figure:** Effect of avibactam (4mg/L) on the activity of ceftazidime against 334 isolates of *P. aeruginosa* collected from cystic fibrosis patients.

**A:** Cumulative MIC distribution with indication of MIC$_{50}$, MIC$_{90}$ and percentage of susceptibility according to the interpretive criteria of EUCAST (S ≤ 8 mg/L; R > 8 mg/L) and CLSI (S ≤ 8 mg/L; R > 16 mg/L). The dotted line points to the limit between susceptible and resistant strains according to EUCAST.

**B:** Reduction in the MIC ($\pm$ SD) of ceftazidime (expressed in number of dilutions) when combined to avibactam as a function of the ceftazidime MIC. The data were used to fit a log Gaussian equation ($R^2 = 0.979$) allowing to calculate that the maximal amplitude of change (no. of dilutions; $4.3 \pm 0.14$) occurred for an MIC of $229 \pm 29$ mg/L.

**C:** Correlation between MICs of ceftazidime alone and ceftazidime/avibactam for each individual strain in the collection using quantile density contour analysis. Colours (from warm [red] to cold [blue]) are indicative of the number of strains for each MIC combination. The dotted lines point to the MIC value above which the isolates are considered resistant strains according to EUCAST interpretive criteria and the figures indicate the percentage of strains in each quadrant.
**A**

<table>
<thead>
<tr>
<th>EUCAST</th>
<th>CLSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>% S / R</td>
<td>% S / I / R</td>
</tr>
<tr>
<td>CAZ</td>
<td>32 / 512</td>
</tr>
<tr>
<td>CAZ-AVI</td>
<td>4 / 64</td>
</tr>
</tbody>
</table>

**B**

Fold reduction in MIC in combination with avibactam

**C**

Contour plot of log₂ ceftazidime MIC vs. log₂ ceftazidime/avibactam MIC.