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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 is 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 β-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 β-lactams may help to alleviate this burden. Ceftazidime is a well-established cephalosporin (on the WHO List of Essential Medicines) with an excellent safety profile and an antibacterial spectrum that includes *P. aeruginosa*. However, ceftazidime is degraded by many β-lactamases, including ESBLs (Ambler classes A and D), cephalosporinases (Ambler class C) and carbapenemases. Avibactam (formerly NXL-104) is a novel non-β-lactam, broad-spectrum β-lactamase inhibitor, with promising inhibitory activity against Ambler class A (including ESBLs and *Klebsiella pneumoniae* carbapenemases), C and D β-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 and 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 diagnosis of CF equally distributed between four European countries with a predominance of recent isolates [Belgium (2010), France (1996–2012), Germany (2012) and the UK (2006–09)] and used them to assess the activity of ceftazidime alone or combined with avibactam. MICs were determined by microdilution in cation-adjusted Mueller–Hinton broth following the CLSI methodology for ceftazidime alone (procured as Glazidim®, the commercial product registered in Belgium for parenteral use; potency, 88.2%; GlaxoSmithKline; Genval, Belgium) and combined with 4 mg/L avibactam (NXL-104, potency 91.7%, batch number 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 for individual strains were assessed using quantile density contour analysis (JMP® version 10.0.2, SAS Institute Inc., Cary, NC, USA). Figure 1(a) shows that isolates in this collection had a high MIC_{90} of ceftazidime (512 mg/L), with only 36% being clinically susceptible (MIC $\leq$8 mg/L) according to EUCAST or CLSI interpretive criteria. When combined with avibactam, the proportion of susceptible strains increased to 76% and the MIC_{90} decreased to 64 mg/L. Figure 1(b) 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 1–128 mg/L range (0.6 is the slope value of a linear regression relating the $\log_2$ MIC of the combination to the $\log_2$ MIC of ceftazidime in that range; $R^2 = 0.965$), 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 ~4 dilutions in MIC for strains for which the ceftazidime MIC was ~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 Figure 1(c), 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 for which the MIC was <256 mg/L. In accordance with the conclusion drawn from Figure 1(c), the ceftazidime MIC was now only 4–8 mg/L for most of the affected

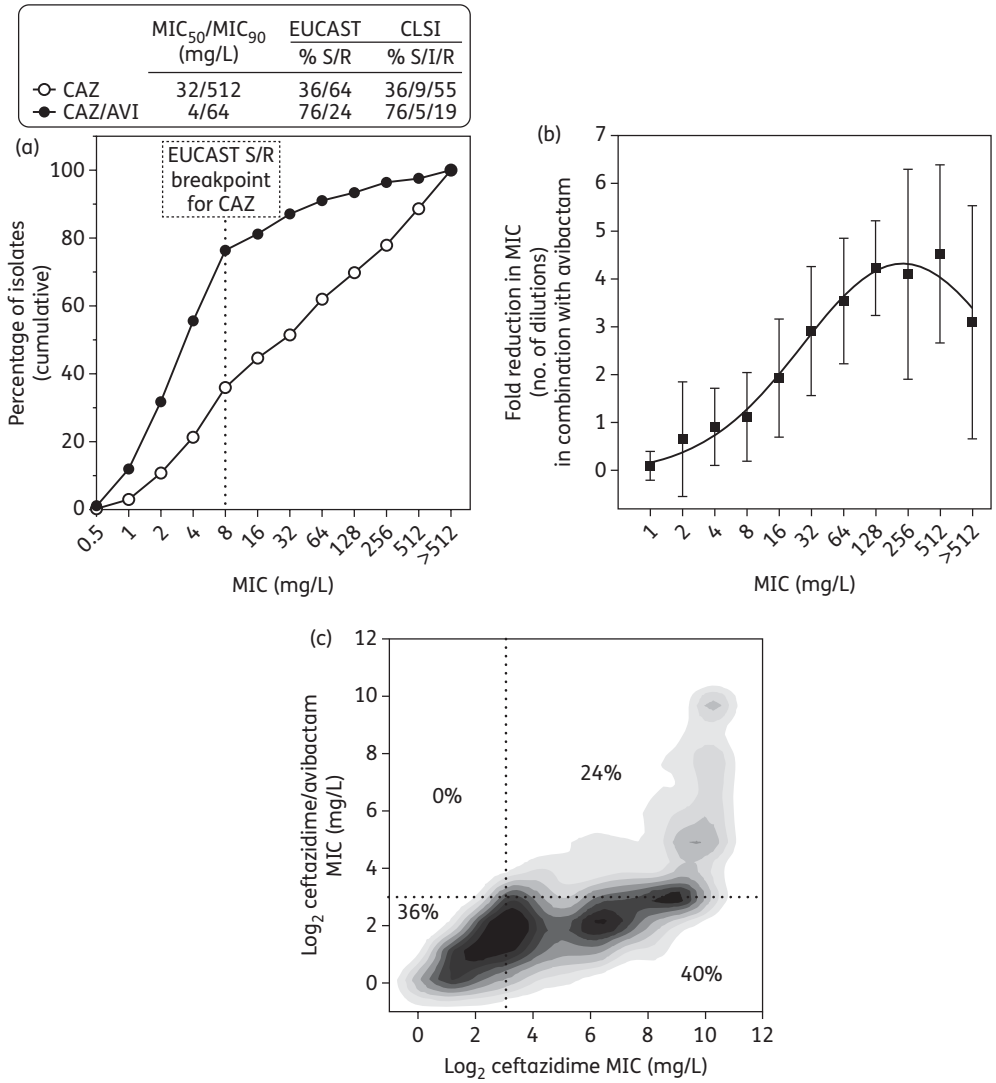

Figure 1. Effect of avibactam (4 mg/L) on the activity of ceftazidime against 334 isolates of *P. aeruginosa* collected from CF patients. (a) Cumulative MIC distribution with indication of MIC_{50}, MIC_{90} and percentage susceptibility according to the interpretive criteria of EUCAST (susceptible, ≤8 mg/L; resistant, >8 mg/L) and CLSI (susceptible, ≤8 mg/L; resistant, ≥32 mg/L). The broken line indicates the limit between susceptible and resistant strains according to EUCAST. (b) Reduction in the MIC (±SD) of ceftazidime (expressed as the number of dilutions) when combined with avibactam as a function of the ceftazidime MIC. The data were used to fit a log Gaussian equation (R^2=0.979) allowing us to calculate that the maximum amplitude of change (number of dilutions, 4.3±0.14) occurred at an MIC of 229±29 mg/L. (c) Correlation between MICs of ceftazidime alone (abcissa) and ceftazidime/avibactam (ordinate) for each individual strain in the collection using quantile density contour analysis. The intensity of each zone (from deep black to light grey) is indicative of the proportion of strains (from large to small) with MICs at the corresponding coordinates. The broken lines point to the MIC value above which the isolates are considered resistant strains according to EUCAST interpretive criteria for ceftazidime and the figures indicate the percentage of strains in each quadrant. AVI, avibactam; CAZ, ceftazidime; S, susceptible; I, intermediate; R, resistant.

strains, a value that is below the EUCAST and CLSI susceptibility breakpoints, extending to CF *P. aeruginosa* isolates the conclusions obtained for pseudomonal isolates of other origins[7] and for other Gram-negative bacteria.[8]

Taken together, these data highlight 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 into the susceptible range those *P. aeruginosa* strains with a ceftazidime MIC ≤256 mg/L.

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