Multidrug-resistant pulmonary infection in cystic fibrosis – what does ‘resistant’ mean?

The threat of multidrug-resistant pathogens is increasingly highlighted (McGowan, 2006) and exacerbated by the disappointing lack of new antimicrobials from post-genomic biotechnology, and the perception that the pharmaceutical industry has lost the appetite for antibiotic development (Spellberg et al., 2004). But what do we mean by ‘resistant’, and, for some infections, should conventional methods of susceptibility testing be revised?

A primary aim of susceptibility testing, by whatever method, is to assess the in vitro susceptibility of bacterial pathogens to a single agent, or combination of agents, in order to provide optimum antimicrobial therapy in vivo. In acute bacterial infections (such as bacteraemia), this usually requires bacterial eradication. Traditionally, a bacterial pathogen is described as resistant if the MIC of an antibiotic exceeds that of a discriminatory breakpoint concentration. Breakpoint concentrations per se are based on clinical, pharmacological and microbiological factors including non-toxic concentrations that are achieved by parenteral administration (MacGowan & Wise, 2001). For most acute infections, susceptibility testing provides a reliable and convenient means of forecasting clinical efficacy. In chronic pulmonary infections, however, such as those found in individuals with cystic fibrosis (CF), the correlation between in vitro and in vivo results is usually poor; thus conventional methods for susceptibility testing are unreliable, and definitions of resistance based on present breakpoints can be misleading.

In this issue of the journal, Falagas and colleagues provide a provocative review which challenges current definitions of multidrug- and pan-resistant pathogens (Falagas et al., 2006). Another recent article describes the disappointing outcome of a large randomized controlled trial to investigate the potential of multiple combination bactericidal antibiotic testing (MCBT) in the treatment of multiresistant pathogens in patients with CF (Aaron et al., 2005). These articles highlight two important components of antimicrobial therapy: Falagas and colleagues focus on definitions derived primarily from in vitro data on antibiotic susceptibility; Aaron and colleagues focus on the clinical value of different methods of susceptibility testing, including combination testing. This editorial was prompted by other important issues in antibiotic therapy which are exemplified in the treatment of chronic pulmonary infection in CF. First: the need to redefine the term ‘resistant’ as a consequence of the development and use of aerosolized and dry powder formulations of existing antimicrobial agents. Second: the problem presented by the high bacterial densities and microbial adaptation characteristic of chronic CF airway infection.

The microbiology and treatment of pulmonary infections in CF is idiosyncratic and challenging. One of the most important and contentious issues in clinical management of CF is the value of susceptibility testing and the most appropriate method to employ. Bacterial resistance can be defined reasonably well in non-CF infections; the situation in lung disease is less clear. Thus Aaron and colleagues are to be congratulated on their excellent, much-needed Canadian investigation into a notoriously complex area. They reported that MCBT did not result in better clinical and bacteriological outcomes in CF pulmonary infection compared to therapy directed by conventional culture and sensitivity testing (Aaron et al., 2005). In a commentary, Smyth (2005) stated that the results of the Canadian study would disappoint many CF clinicians and their patients. He could also have added microbiologists to this list! Smyth also suggested that susceptibility testing of antibiotic combinations might be useful if testing was used strategically to control the emergence of resistance, stating that ‘CF clinicians must be tough on resistant infection and tough on the causes of resistance’. These articles emphasize the poignant lesson that there is still much we don’t understand about the microbiology and treatment of CF pulmonary infections. For example, how do we define ‘resistant’ in a situation when conventional methods for susceptibility testing do not predict clinical outcome?

Current perception of antibiotic resistance is primarily based on breakpoint concentrations achieved by parenteral administration, traditional pharmacological models of drug distribution, and homogeneous populations of in vitro-grown planktonic (free-swimming) bacteria. In the context of chronically infected CF Airways, this view of bacterial behaviour is inappropriate. In the early 1980s, the seminal paper by Lam and colleagues (Lam et al., 1980) preceded further compelling evidence that in CF Airways, mucoid, alginate-producing Pseudomonas aeruginosa grow as bacterial microcolonies within complex biofilms (Govan & Deretic, 1996; Stewart & Costerton, 2001). The protective properties and architecture of Pseudomonas biofilms in CF Airways result from a combination of bacterial alginate, neutrophil DNA and respiratory mucin (Govan & Deretic, 1996; Stewart & Costerton, 2001). The protective effects of biofilms on antibiotic action are also multifactorial, and result from a combination of reduced antibiotic penetration, reduced bacterial growth rate, the high ionic content of alginate and phenotypic variation (Drenkard & Ausubel, 2002). Thus bacterial biofilms created in
plastic microtitre plates may not be a sufficient model to mimic the bacterial physiology and milieu of CF airways. Clinical trials may show that bacterial susceptibility based on biofilm-based testing does not predict clinical efficacy; however, resistance under such conditions may predict resistance within CF airways.

In a further attempt to explain their results, Aaron and colleagues suggested that non-bactericidal effects of antibiotic therapy on bacterial virulence may play a role in clinical response in patients with CF pulmonary exacerbations. There is strong in vitro evidence to support this hypothesis (Govan, 2002). However, their results could also be explained by the comparison of MCBT with ‘conventional susceptibility testing methods’, which creates the potential for variability in the tests performed in the different participating laboratories. Numerous studies have shown that the results of ‘conventional’ susceptibility testing of *P. aeruginosa* can vary depending on the methodology used, and that the optimal method for antibiotic susceptibility testing of mucoid *P. aeruginosa* in particular is unclear (Saiman et al., 1999; Burns et al., 2001; Smith et al., 2003; Morosini et al., 2005; Sader et al., 2006).

Another factor which could affect the reliability of conventional susceptibility testing is the presumption that clinical efficacy can be predicted based on only a tiny sample of the high bacterial densities found in infected CF airways. Susceptibility testing, by whatever method, is usually performed on less than 10 c.f.u. cultured from a single specimen. This represents only a ‘moment of infectious time’ and an optimistic but ‘statistically naïve’ sample of the $10^9$ c.f.u. ml$^{-1}$ and striking phenotypic diversity of *P. aeruginosa* within CF airways. In established infection, multiple colonial morphotypes of *P. aeruginosa* can be cultured from a single CF sputum. In some laboratories, this problem is addressed by testing representatives of different morphotypes. Unfortunately, susceptibility patterns can differ even when c.f.u. of the same morphotype and strain of *P. aeruginosa* are tested (Fyfe & Govan, 1984; Foweraker et al., 2005).

The future of antibiotic therapy against multidrug-resistant CF pathogens is uncertain. As the supply of new antibiotics continues to slow, those agents which reach the market are expensive, including new formulations of existing agents. The study by Aaron et al. (2005) and the perceptive commentary by Smyth (2005) emphasize the urgent need to find methods of susceptibility testing which more closely mimic the infectious process, and allow more reliable predictions of clinical efficacy. These articles, and the challenging review by Falagas et al. (2006), also emphasize the need to redefine ‘resistance’ terminology according to the pathogens concerned, their resistance mechanisms, the pathophysiology of pulmonary infection and the therapeutic end points to be achieved. In CF pulmonary infection, the latter are important considerations. For example, is antibiotic therapy being used at first isolation to eradicate the pathogen and delay early infection, or to provide a palliative role in chronic infection by reducing bacterial virulence and inflammation? Further studies may show that conventional susceptibility tests may be appropriate to treat early infection. However, treatment of chronic CF pulmonary infection poses the particular, and perhaps unique, challenge of high bacterial densities (and hence sampling problems), variable antibiotic sensitivity within a single strain, biofilm growth, slow growth rate, anaerobiosis, bacterial surface changes, hypermutator status and quorum sensing acting together within a complex biological milieu. In the treatment of chronic *P. aeruginosa* in CF patients, the term ‘resistant’ should take account of the complex pathophysiology of these infections and the high concentrations of antibiotics which can be achieved in the infected airways by inhaled antibiotics (Burns et al., 1999; Morosini et al., 2005).

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