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## Early *Pseudomonas aeruginosa* infection in individuals with cystic fibrosis: is susceptibility testing justified?

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**Objectives:** To test the presumption that *Pseudomonas aeruginosa* isolates responsible for initial lung infection in individuals with cystic fibrosis (CF) are invariably susceptible to antipseudomonal agents.

**Methods:** Antibiotic susceptibility was determined (MIC and Etest) in two populations of *P. aeruginosa* associated with initial lung infection. Population 1: environmental isolates ( $n=78$ ). Population 2: clinical isolates responsible for first infection in previously non-infected patients (85 isolates from 85 patients). Susceptibility or resistance was determined using current BSAC guidelines; ninth version (2009).

**Results:** The majority ( $\geq 90\%$ ) of isolates in both bacterial populations were susceptible to the front-line antipseudomonal agents; colistin, ciprofloxacin, tobramycin, ceftazidime, amikacin and meropenem. Up to 10% of isolates were resistant to one or more antibiotics. A single isolate from each population would be defined as resistant to tobramycin based on a breakpoint ( $>128$  mg/L) that has been suggested for use in patients receiving inhaled therapy.

**Conclusions:** The high prevalence of susceptibility found in *P. aeruginosa* isolates associated with initial infection contrasts with the high prevalence of resistance found in isolates from chronic CF lung infection. However, susceptibility in early isolates cannot be presumed. Until further data are obtained from clinically based studies, susceptibility tests should continue to be performed to assist the choice of antibiotics for treatment of early infection.

**Keywords:** antibiotics, environmental, Etest, resistance

### Introduction

*Pseudomonas aeruginosa* is the major bacterial pathogen responsible for pulmonary failure in individuals with cystic fibrosis (CF). Antibiotic therapy against *P. aeruginosa* plays an essential role in the management of three stages of infection. First, in early infection to prevent or delay transition to chronic infection; second, in chronic infection to maintain lung function by reducing pulmonary inflammation; and third, to treat acute pulmonary exacerbations. Evidence that conventional antibiotic susceptibility tests, including combination testing, are poorly predictive of clinical outcome in chronic infection challenges the justification for routine *in vitro* susceptibility testing.<sup>1–3</sup>

In a 5 year double-blind multicentred study, Aaron *et al.*<sup>2</sup> compared antibiotic choice for treatment of acute exacerbations, using conventional susceptibility tests, with that based on multiple combination bactericidal testing. Disappointingly, the study showed no difference in treatment outcome. Limitations of this study were that susceptibility was based on previous sputum isolates analysed within 3 months of the exacerbation, and that

participating centres used their own methodologies (not described) for sputum culture and susceptibility testing. The study also relied on local investigators to select isolates of *P. aeruginosa* for testing. The issue of sampling is vital since variability in susceptibility between isolates of *P. aeruginosa* from the same sputum, and even with the same colony morphotype, is characteristic of chronic *P. aeruginosa* infection in CF.<sup>3,4</sup>

To date, concerns that conventional and biofilm-associated antibiotic susceptibility tests do not predict clinical outcome have focused on treatment of chronic *P. aeruginosa* infection and associated and recurring pulmonary exacerbations. It is not clear whether similar doubts should also apply to treatment of early infection, which plays an increasingly important role in clinical management following the early diagnosis of CF through national newborn screening programmes. In the absence or failure of early therapy, *P. aeruginosa* within the CF lung evolves genomically and phenotypically over many years to chronic infection, at which stage eradication is seldom achieved. To our knowledge, there is no evidence that early isolates of *P. aeruginosa* share the adaptation and phenotypic

heterogeneity associated with chronic infection. It is also unclear whether the lack of clinical value of susceptibility testing during chronic infection also applies to treatment of early infection.

Current knowledge of the susceptibility of CF isolates of *P. aeruginosa* is primarily based on chronic infection or acute exacerbations. If early isolates are invariably susceptible to antipseudomonal agents, the choice of antibiotics could be prescriptive and made without recourse to the expense and time-consuming process of susceptibility testing.

To test the presumption that *P. aeruginosa* isolates responsible for initial infection of CF individuals are invariably susceptible to front-line antipseudomonal agents, we investigated the *in vitro* susceptibility of two populations of *P. aeruginosa* associated with early infection. Population 1 comprised *P. aeruginosa* from natural environments. With the exception of sibling-to-sibling transfer or epidemic spread, these are considered to be the major source of early infection. Population 2 comprised clinical isolates responsible for the first infection of CF patients.

## Materials and methods

### Bacteria

Population 1 comprised 78 environmental isolates of *P. aeruginosa* from hospital and non-hospital environments (20 isolates and 58 isolates, respectively); sites included sinks, drains, vegetables and ponds. Identification was based on colony morphology, blue-green pigmentation and a positive oxidase reaction. Presumptive non-pigmented isolates were identified using API 20NE kits (bioMérieux, Marcy l'Étoile, France) and species-specific PCR.<sup>5</sup> Population 2 comprised 85 first *P. aeruginosa* sputum isolates obtained during longitudinal studies from 85 CF patients attending CF centres in the UK and Tuscany. Patients, aged 2 months to 40 years, had previously been *Pseudomonas* free and had not received antipseudomonal treatment. All isolates examined exhibited a non-mucoid colony morphotype and were selected for in the same manner using Difco *Pseudomonas* isolation agar.

Within each population, clonality was excluded by PFGE. PFGE and strain-specific PCR were also used to exclude known epidemic strains,

in particular the widespread Liverpool epidemic strain, LES. To exclude heterogeneity in susceptibility in first isolates from the same sputum, we examined a subset of 60 multiple colonies; these comprised 10 clonally related colonies picked prospectively from the original sputum samples from six patients.

### Antibiotic susceptibility: MIC and Etest strips

The susceptibility of the two *P. aeruginosa* strain panels and multiple colony subset to front-line antipseudomonal agents, namely colistin, ciprofloxacin, tobramycin, ceftazidime, amikacin and meropenem, was determined. MICs were determined by Etest (bioMérieux UK Ltd, Basingstoke, UK) according to the manufacturer's instructions. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as controls for the potency and breakpoints of the antibiotics used. Revised BSAC breakpoints (Table 1) for *Pseudomonas* species were used to determine resistance as follows: colistin, >2 mg/L; ciprofloxacin, >1 mg/L; ceftazidime, >8 mg/L; tobramycin, >4 mg/L; amikacin, >16 mg/L; and meropenem, >8 mg/L.

## Results

The degree of variability encountered for the ATCC control strains and the *P. aeruginosa* panels was within the error of the test and in no instance involved a change from susceptible to resistant, or vice versa. Analysis of MICs showed that the majority ( $\geq 90\%$ ) of isolates from environmental sites, and isolates responsible for initial *P. aeruginosa* infection of CF patients, were susceptible to each of the antipseudomonal antibiotics. However, according to current breakpoints, up to 10% of isolates in both *Pseudomonas* strain panels exhibited resistance to at least one antibiotic. This included high-level (>256 mg/L) resistance to colistin in an environmental isolate whose identification was confirmed.

Molecular typing indicated that sibling-sibling transfer or clonality did not account for any of the resistance observed. There was no significant difference in susceptibility between the two panels of isolates to any of the antibiotics tested ( $\chi^2$  analysis,  $P=0.182$ ). Multiresistance to two or more antibiotic classes was rare. Interestingly, in both bacterial populations, we observed an

**Table 1.** Percentages of susceptible *P. aeruginosa* isolates and MIC ranges (mg/L) for environmental and first CF isolates

Antimicrobial (resistance breakpoint) <sup>a</sup>		Environmental isolates (n=78)	First CF isolates (n=85)
Colistin (>2 mg/L)	% susceptible	96	98
	MIC range	0.25 to >256	1-4
Ciprofloxacin (>1 mg/L)	% susceptible	96	96
	MIC range	0.094-8	0.064 to >256
Ceftazidime (>8 mg/L)	% susceptible	95	95
	MIC range	0.5 to >256	0.75 to >256
Tobramycin (>4 mg/L)	% susceptible	92	90
	MIC range	0.38 to >256	0.38 to >256
Amikacin (>16 mg/L)	% susceptible	97	93
	MIC range	1 to >256	1 to >256
Meropenem (>8 mg/L)	% susceptible	93	98
	MIC range	0.032 to >32	0.064-16

<sup>a</sup>Based on BSAC guidelines, ninth version, 2009.

isolate that would be defined as resistant to tobramycin based on Etest and a breakpoint (>128 mg/L) that has been suggested for predicting *P. aeruginosa* susceptibility to inhaled tobramycin.<sup>6</sup> In contrast to susceptibility testing of *P. aeruginosa* associated with chronic infection, and outside the error of the test, examination of multiple isolates from six individual sputa showed no evidence of heterogeneity in the MICs for clonal isolates sampled from a single sputum (data not shown).

## Discussion

The microbiology of lung disease in patients with CF is complex. Challenges include the need to explain why *in vitro* susceptibility frequently fails to predict clinical efficacy, and the need to appreciate that the terms susceptible and resistant are ill-defined for chronic *P. aeruginosa* infections in individuals with CF. In particular, conventional definition of resistance based on breakpoints, serum drug concentrations, *in vitro* MICs and parenteral therapy does not apply to CF lung infections treated with inhaled antibiotics.<sup>4</sup>

There has also been increasing interest in the cost-effectiveness of antibiotic treatment for the different stages of *P. aeruginosa* infection in CF. These studies have focused on the costs of delivering antibiotics to CF patients either as outpatients or in hospital; to our knowledge, the additional costs of susceptibility testing have not been considered. However, in one large UK CF clinic, a reduction in the number of routine susceptibility tests performed in cases of chronic *P. aeruginosa* resulted in an annual saving on consumables and staff time of 10000 Euros without impact on short-term clinical outcomes.<sup>7</sup>

The present debate on the justification for routine susceptibility testing in the management of CF lung infection has focused on *P. aeruginosa* isolates responsible for chronic infection and pulmonary exacerbations. The major objective of our study was to test the presumption held by some clinicians and microbiologists that *P. aeruginosa* populations responsible for early infection are intrinsically susceptible to antipseudomonal agents. If our study proved this presumption correct, it would provide preliminary evidence that routine susceptibility testing might also be reduced in the management of early infection. To our knowledge, such an investigation has not been reported. One reason could be the challenge of collecting and characterizing the necessary *P. aeruginosa* strain panels critical for this study, and in particular the panel of CF isolates responsible for initial infection that was made possible by longitudinal studies of individual patients.

In our study, the *in vitro* susceptibility of *P. aeruginosa*, isolated from environmental sites, or isolates responsible for initial infection in CF patients, was reassuring, and with the exception of colistin, contrasts with the disturbingly high resistance to antipseudomonal agents reported in previous surveys.<sup>8</sup>

Although the majority of *P. aeruginosa* associated with initial infection show *in vitro* susceptibility to front-line antipseudomonal agents, our results indicate that susceptibility cannot be presumed. Two large multicentre studies focused on antibiotic therapy of early *P. aeruginosa* infection merit consideration. Interestingly, the recently published European ELITE study did not address the potential role of susceptibility/resistance as a reason for success or failure in bacterial eradication.<sup>9</sup> Also, although the North American EPIC study may provide data on

the emergence of antibiotic resistance, it does not include an assessment of the validity of susceptibility testing.<sup>10</sup>

Clinical studies focused on early eradication of *P. aeruginosa* are necessary to determine whether susceptibility testing performed by conventional, biofilm-based methods or by a recently described susceptibility breakpoint index<sup>8</sup> correlate with clinical outcomes, and to identify breakpoints that take account of the high antibiotic concentrations achieved by aerosol delivery. Until such information is available, it would seem prudent to continue susceptibility testing of *P. aeruginosa* responsible for early infection in individuals with CF.

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## Transparency declarations

None to declare.

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