Variable antibiotic susceptibility in populations of *Pseudomonas aeruginosa* infecting patients with bronchiectasis

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Received 24 October 2008; returned 23 November 2008; revised 2 January 2009; accepted 4 January 2009

**Objectives:** To investigate variability in colony morphology and antibiotic susceptibility in populations of *Pseudomonas aeruginosa* from sputa of patients with bronchiectasis without cystic fibrosis (CF) compared with *P. aeruginosa* isolated from patients with CF, and from other infections as controls.

**Methods:** *P. aeruginosa* was cultured from 31 patients with non-CF bronchiectasis, 24 with CF, 7 ventilated patients and 9 skin swabs. Four colonies of each morphotype of *P. aeruginosa* were tested for susceptibility to 12 antibiotics by disc diffusion. The variability in susceptibility between the isolates in each patient’s population of *P. aeruginosa* was investigated.

**Results:** The classic morphotype of *P. aeruginosa* was cultured from control samples with an average variation in zone size of 2 mm (range 0–4 mm) for the four colonies tested. Non-CF bronchiectasis sputa contained 1–3 colonial morphotypes of *P. aeruginosa*; the average difference between the largest and smallest zone sizes found in all examples of the morphotypes present in each sample varied from 3 mm (1–9 mm) for colistin to 8 mm (0–24 mm) for piperacillin/tazobactam. CF sputa contained 2–6 morphotypes of *P. aeruginosa* with a wider variation of susceptibility. There was variation between bacteria of the same morphotype from non-CF bronchiectasis and CF sputa.

**Conclusions:** Phenotypic variation in colonial form and antibiotic susceptibility is not unique to chronic infection in CF but is also found in non-CF bronchiectasis. This questions the use of current susceptibility testing methods for the complex populations of bacteria found in chronic lung infection.

Keywords: *P. aeruginosa*, morphotypes, disc diffusion, cystic fibrosis

**Introduction**

Bronchiectasis is a chronic lung condition with dilatation of the bronchi, cough and chronic sputum production. While in some patients it results from a range of congenital or acquired conditions, including childhood infection, airway obstruction or immune defect, many cases are idiopathic. More is known about the microbiology of infection in cystic fibrosis (CF) than in non-CF bronchiectasis, but the latter is more prevalent and causes considerable morbidity. Patients have chronic respiratory infection with intermittent exacerbations associated with the worsening of symptoms.¹ The sputum in non-CF bronchiectasis may be chronically infected with bacterial pathogens, most commonly non-typeable *Haemophilus influenzae* (NTHi) or *Pseudomonas aeruginosa*. Patients infected with *P. aeruginosa* are more likely to require hospitalization and have a reduced quality of life than those infected with NTHi.² *P. aeruginosa* is inherently resistant to many antibiotics and easily acquires resistance.³ Reliable detection of antibiotic resistance may therefore be important for treatment of acute exacerbations in patients with chronic lung disease.

*P. aeruginosa* in CF bronchiectasis has been well characterized and chronic infection is associated with phenotypic changes, for example, loss of motility, defective lipopolysaccharide, alginate production (mucoid phenotype), auxotrophy and genetic divergence.⁴ These characteristics may vary between bacteria in sputum and varying antibiotic susceptibility is also seen.⁵ Far less is known about *P. aeruginosa* infection in non-CF bronchiectasis. We therefore compared the variability of antibiotic susceptibility and range of morphotypes of *P. aeruginosa* in sputa of patients with non-CF bronchiectasis with *P. aeruginosa* in CF sputa. As controls, we tested *P. aeruginosa* from acute lung infection in intensive care unit patients and skin swabs from leg ulcers.
Table 1. Variation in susceptibility of *P. aeruginosa* isolated from non-CF bronchiectasis, CF and other (control) clinical samples

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Control samples, <em>n</em> = 16</th>
<th>Non-CF bronchiectasis sputa, <em>n</em> = 33</th>
<th>CF sputa, <em>n</em> = 24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (range) zone size difference (mm)</td>
<td>number of samples where susceptibility of isolates varied between S and R</td>
<td>mean (range) zone size difference (mm)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1 (0–4)</td>
<td>0</td>
<td>5 (0–18)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>2 (1–4)</td>
<td>4</td>
<td>8 (0–18)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>2 (0–4)</td>
<td>0</td>
<td>6 (1–17)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2 (1–4)</td>
<td>0</td>
<td>7 (2–24)</td>
</tr>
<tr>
<td>Colistin</td>
<td>1 (0–2)</td>
<td>0</td>
<td>3 (1–9)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2 (0–3)</td>
<td>0</td>
<td>5 (0–17)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>2 (1–3)</td>
<td>0</td>
<td>6 (0–23)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>2 (1–4)</td>
<td>0</td>
<td>8 (1–20)</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>1 (0–3)</td>
<td>0</td>
<td>6 (1–22)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>2 (0–4)</td>
<td>0</td>
<td>7 (1–25)</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>2 (0–4)</td>
<td>0</td>
<td>8 (0–24)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>2 (0–4)</td>
<td>0</td>
<td>6 (0–21)</td>
</tr>
</tbody>
</table>

Four colonies of each morphotype present in each sample were separately tested for susceptibility to 12 antibiotics. The zone size difference is the difference in millimetres between the largest and smallest zone size obtained from all colonies tested from each sample.
Methods

*P. aeruginosa* was cultured by standard methods from the following:

(i) Thirty-three sputa from 31 patients with well-characterized non-CF bronchiectasis, mean age 63 years (range 38–76 years); 23 sputa were from bronchiectasis patients when stable and 10 at acute exacerbation. Two patients provided sputum when stable and at exacerbation.

(ii) Twenty-four sputa from adult CF patients at acute exacerbation, mean age 21 years (range 16–32 years).

(iii) Six sputa and one tracheostomy swab from ventilated patients without bronchiectasis and nine skin swabs from leg ulcers (control samples).

Morphotypes were counted from primary growth on PCFC agar (pseudomonas agar base with cetrimide, fusidic acid and cefaloridine selective agar supplement; Oxoid Ltd, Basingstoke, UK) after incubation for 48–72 h at 37°C in air. Defining characteristics of morphotypes included size, texture, colour and mucoidity. Four colonies of each morphotype were separately tested for susceptibility to 12 antibiotics by the BSAC standardized disc susceptibility method as previously described. All tests were performed by the same individual. The diameter of the zone of inhibition was measured using electronic callipers and the difference in millimetres between the largest and smallest zone sizes obtained for all colonies tested from each sample was calculated for each antibiotic. This measurement was termed the zone size difference.

**Figure 1.** Mean zone size difference, the difference in millimetres between largest and smallest zone size obtained for all colonies of *P. aeruginosa* tested from each sample for each antibiotic, with 95% confidence interval (CI). AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; COL, colistin; GEN, gentamicin; IPM, imipenem; MEM, meropenem; NET, netilmicin; PIP, piperacillin; TZP, piperacillin/tazobactam; TOB, tobramycin.
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Results

One hundred and sixty isolates of *P. aeruginosa* were studied. All control samples contained a single morphotype, in all cases of classic, rough appearance. One morphotype was present in 18 non-CF bronchiectasis sputa, two morphotypes in 13 sputa and three in 2 sputa. These included rough, mucoid, smooth and dwarf forms. The 23 sputa from patients with stable bronchiectasis had a total of 37 morphotypes and the 10 sputa from acute exacerbation had 13 morphotypes. CF sputa contained, on average, four morphotypes (range 2–6).

Table 1 shows the variation in susceptibility for *P. aeruginosa* isolated from each sample type. For each antibiotic, the mean (range) zone size difference is shown together with the number of samples in which the antibiotic breakpoint between susceptible and resistant was crossed. This breakpoint was crossed in 4 of the control samples (for aztreonam only), in 11 of the non-CF bronchiectasis samples (affecting up to seven antibiotics per sample) and in 23 of the CF samples (up to nine antibiotics per sample). Variation in inhibition zone size was seen between multiple colonies of the same morphotype for both mucoid and non-mucoid colony forms. Figure 1 shows the mean zone size difference and 95% confidence interval according to the sample type.

Discussion

This is the first study in non-CF bronchiectasis to look at the variation in antibiotic susceptibility of *P. aeruginosa*. We used disc diffusion susceptibility testing, the most common method in UK clinical laboratories. While *P. aeruginosa* from control samples showed fairly uniform zone sizes, those from non-CF bronchiectasis showed variation in morphotype and susceptibility similar to, but less marked than, that shown by *P. aeruginosa* isolated from CF sputa.

The range of zone sizes was not the result of two distinct populations as a wide spread of degrees of susceptibility was observed. It is not clear how such variability develops. Patients with bronchiectasis receive many courses of antibiotics, but these may be expected to select a resistant phenotype rather than a diverse population. Hypermutator strains with a high rate of spontaneous mutations due to defects in DNA repair genes are common in CF and were recently found in a small study of non-CF bronchiectasis. They can generate a range of mutations for antibiotic resistance. In chronic lung infection, *P. aeruginosa* is thought to grow in biofilms within which exists a wide range of environmental conditions. Bacteria growing in each micro-niche may have different growth rates and metabolic adaptations (e.g. anaerobic metabolism) that affect their susceptibility to antibiotics. The concentration of antibiotic may differ in parts of the biofilm due to variable penetration and/or binding. In each niche, therefore, the selection pressure for antibiotic resistance could differ. Expectorated sputum contains biofilm fragments and this may explain why such mixed populations of *P. aeruginosa* are then cultured.

Extremely large zones of inhibition were measured for many bacterium–antibiotic combinations. Hypersusceptible strains of *P. aeruginosa* were described in patients with CF and non-CF bronchiectasis over 30 years ago. These are not only very susceptible to anti-pseudomonal antibiotics but also to drugs not normally considered active, such as ampicillin and trimethoprim. While a recent study has shown that engineered drug efflux mutants are hypersusceptible, there is no literature on the clinical relevance of naturally occurring hypersusceptibility, and this warrants further work.

Current disc diffusion susceptibility testing methodology was developed using isolates from a range of infections but may not be suitable for the heterogeneous populations found in chronic infection in CF and non-CF bronchiectasis. In our study, there were many examples where the range of zone sizes crossed the breakpoint between resistance and susceptibility. Antibiotic susceptibility is therefore likely to be poorly reproducible with the result depending on the particular colonies tested. We have previously shown in CF that recommended methods miss resistance and are poorly reproducible. Alternatives such as the use of media with incorporated antibiotics improve the retrieval of resistant isolates in CF and may be useful in non-CF bronchiectasis.

Antibiotic treatment of exacerbation of chronic infection in CF and non-CF bronchiectasis is clinically effective but may not clear the infection. The relevance of *in vitro* resistance to the clinical effectiveness of antibiotics has been questioned in CF. It has been suggested that testing bacteria in biofilms may be more relevant than current methods based on planktonic growth. More work is needed to optimize antibiotic susceptibility testing in these diverse populations of bacteria infecting the damaged lung.

Acknowledgements

Part of this work was presented at the British Thoracic Society Winter Meeting (London, UK, 2007: Oral Presentation S127).

We thank Carol Freeman, R&D Unit, Papworth Hospital for assistance with data.

Funding

The work was partly supported by grants from the UK Cystic Fibrosis Trust and the Evelyn Trust.

Transparency declarations

None to declare.

References


