Microbiologic follow-up study in adult bronchiectasis

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**Summary**
There is minimal published longitudinal data about pathogenic microorganisms in adults with bronchiectasis. Therefore a study was undertaken to assess the microbiologic profile over time in bronchiectasis.

A prospective study of clinical and microbiologic outcomes was performed. Subjects were assessed by a respiratory physician and sputum sample were collected for analysis. Subjects were followed up and had repeat assessment performed.

Eighty-nine subjects were followed up for a period of 5.7 ± 3.6 years. On initial assessment the two most common pathogens isolated were *Haemophilus influenzae* (47%) and *Pseudomonas aeruginosa* (12%) whilst 21% had no pathogens isolated. On follow-up review results were similar (40% *H. influenzae*, 18% *P. aeruginosa* and 26% no pathogens). The prevalence of antibiotic resistance of isolates increased from 13% to 30%. Analysis of a series of *H. influenzae* isolates showed they were nearly all nontypeable and all were different subtypes. Subjects with no pathogens isolated from their sputum had the mildest disease, while subjects with *P. aeruginosa* had the most severe bronchiectasis.

Many subjects with bronchiectasis are colonized with the same bacterium over an average follow-up of 5 years. Different pathogens are associated with different patterns of clinical disease.

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**Introduction**
Bronchiectasis remains a prevalent respiratory disease. It has been estimated that there are more than 110,000 adults in the US who have bronchiectasis.1 In addition it overlaps with chronic obstructive pulmonary disease (COPD) and two recent studies have found that 29%2 and 50%3 of subjects...
diagnosed with COPD have evidence on bronchiectasis on high resolution computed tomography scanning (HRCT).

Bronchiectasis is characterized by chronic airway infection resulting in significant clinical disease and impaired lung function. There have been a large number of studies that have assessed factors involved in the aetiology of bronchiectasis. However, there is relative lack of information available about the microbiology of this condition. In particular, there is minimal longitudinal data about pathogenic microorganisms in bronchiectasis. This information is important as it has implications for the pathogenesis of this condition as well as for appropriate antibiotic selection.

To examine the microbiologic profile in bronchiectasis, a prospective study of a cohort of adult patients with bronchiectasis was performed.

Methods

Patient cohort

The patient cohort consisted of adults who were referred by their family doctors for assessment at Monash Medical Centre, a University tertiary-referral hospital. Subjects were seen between 1990 and 2004. Bronchiectasis was diagnosed on high-resolution CT scanning by a consultant radiologist using standard criteria.4

Subjects had a detailed clinical assessment performed by a respiratory physician (PK/PH) and a sputum sample was taken when the patient was clinically stable (i.e. patient had not had an exacerbation and had not received antibiotics for at least 1 month). Subjects were screened for underlying causes of bronchiectasis with full blood examinations, immunoglobulins, neutrophil and lymphocyte function, aspergillus precipitins and skin testing, ciliary function and cystic fibrosis mutation analysis. Patients were followed up for a minimum period of 1 year when they were reviewed again by a respiratory physician (PK/PH) and asked to produce another sputum sample (when they were clinically stable) for microbiological analysis. A total of 135 patients were screened of whom 89 subjects were able to produce sputum samples suitable for microbiological analysis on initial and follow-up review.

This project was approved by the Human Ethics Committee of Monash Medical Centre and informed consent was obtained from all patients.

Collection of sputum samples

Patients were asked to perform chest physiotherapy for a minimum of 5 min prior to the expectoration of sputum. Samples were processed in the Diagnostic Microbiology laboratories of Monash Medical Centre (Southern Health Pathology) if there were >25 leucocytes and <25 epithelial cells per field using a low magnification lens. Specimens were inoculated onto chocolate agar and horse blood agar (50%)/McConkey agar plates and incubated at 35°C in 5% CO2 for 48 h. Specimens were then examined for growth. Specimens for mycobacterial analysis were sent to the Victorian Infectious Disease Reference Laboratory where specimens had Ziehl-Nielsen staining performed and were cultured on Löwenstein-Jensen medium. Negative bacterial cultures were discarded after 7 days, negative cultures for fungi after 4 weeks and Lowenstein-Jensen cultures after 6–8 weeks. Susceptibility testing was performed using National Committee for Clinical Laboratory Standards.5

Sensitivity testing was performed using discs; VITEK (bio-

Merieux, Marcy-l’Etoile, France) and E-test (AB Biodisk, Solna Sweden) for; Haemophilus influenzae and Moxarella. catarrhalis (amoxicillin/erythromycin/tetracycline), Staphylococcus aureus (methicillin), Streptococcus pneumoniae (penicillin) and Pseudomonas/other Gram-negative species (gentamicin/ciprofloxacin).

Analysis for nontypeable H. influenzae

Samples of H. influenzae isolated from sputum samples were collected in the Microbiology Laboratory at Monash Medical Centre and frozen at –70°C in glycerol broth and stored for further analysis. Isolates of H. influenzae were examined for the presence of capsular serotypes (α–f), to assess whether they were typeable or nontypeable. Samples were thawed, inoculated into agar and then taken to the Microbiology Diagnostic Unit in the Department of Microbiology and Immunology at Melbourne University for typing for polysaccharide capsules by using anti-sera α–f and biotyping. NTHi samples were then taken to the Microbiology Research Laboratory in the Department of Microbiology and Immunology at Melbourne University, for outer membrane protein analysis. This was performed by a standardized method using sodium dodecyl sulfate-polyacrylamide gel electrophoresis.6,7

Statistical methods

Results were expressed as mean and standard deviation (SD) or number of patients (n) and percentage (%). Statistical analysis was performed to assess the association between patient factors on initial review (age, sex, sputum volume, hospitalisation in the last year for an exacerbation of bronchiectasis, number of exacerbations per year, number of lobes with bronchiectasis on HRCT, FEV1, and FVC) with the presence of the same microbial isolate on follow-up and antibiotic resistance. Statistical analysis was performed using Stata software (College Station, TX).

Results

The patient cohort was predominantly female (70%) with an average age of 57 ± 14 years. Most subjects (77%) had idiopathic disease. None were current smokers and 18% had a history of previous smoking. Baseline characteristics of the patients are summarised in Table 1.

Sputum isolates in subjects with bronchiectasis

On initial assessment, the most common bacterial isolate was H. influenzae, present in 42 (47%) of the cohort of 89 patients. The next most common bacteria were P. aerugi-

nosa isolated in 11 patients (12%), Moxarella catarrhalis in 7 patients (8%) and S. pneumoniae isolated in 6 (7%). Only 3 patients had Staphylococcus aureus isolated. Two patients
had Aspergillus spp. isolated from their sputum and two had Mycobacterium avium complex. A large number of patients (21%) had no potential pathogenic microorganisms (designated as no pathogens) isolated from their sputum. Results are shown in Table 2.

Follow-up sputum samples were obtained an average period of 5.7 ± 3.6 years after initial review. Overall results were fairly similar to the initial review, with the dominant bacteria isolated being H. influenzae present in 36 patients (40%) and P. aeruginosa isolated in 16 patients (18%). Again there was a large proportion of subjects who had no pathogen isolated from their sputum 23 patients (26%).

A comparison was performed between the results of initial and follow-up sputum samples. The follow-up sputum samples grew the same organism in 50 patients (56%). Of the 42 subjects who had H. influenzae isolated on initial assessment, 27 of these patients (64%) had H. influenzae isolated from their sputum on follow-up review. A similar picture occurred in the 11 subjects with P. aeruginosa isolated on their initial assessment, eight of whom (73%) had Pseudomonas isolated again on follow-up. Logistic regression analysis showed that subjects who had the same isolate on follow-up had a significantly higher number of exacerbations (3.5 ± 1.9 per year) compared with noncolonized subjects (2.7 ± 1.7) (p = 0.04) with an odds ratio of 1.3 (95% confidence interval 1.0, 1.7).

Resistance to antibiotics

Antibiotic susceptibility testing was performed for all isolates. On initial assessment 12 sputum isolates (13% of patient group) showed antibiotic resistance. On follow-up review there was a higher level of resistance with 27 isolates (30%) demonstrating antibiotic resistance (Table 3). Resistance to β-lactams in subjects with H. influenzae, Streptococcus pneumoniae or M. catarrhalis increased from 11% to 26%. Resistance to gentamicin in subjects with P. aeruginosa/other Gram-negative pathogens increased from 14% to 39%.

The characteristics of patients with resistant and sensitive pathogens are compared in Table 4. Patients with resistant bacteria were significantly more likely to have been hospitalised (52% versus 21%; p = 0.006) and have had a greater number of exacerbations (mean [SD] of 4.0 [1.8] versus 2.8 [1.7]; p = 0.007) compared to those with sensitive bacteria. Logistic regression analysis resulted in a
model relating presence of antibiotic resistance to hospitalisation (for an exacerbation of bronchiectasis) and the number of exacerbations. On average, hospitalized patients had an odds ratio (OR) of 3.5 (95% confidence interval [CI] 1.2, 9.6; \( p = 0.017 \)) for the presence of antibiotic resistance compared to nonhospitalized patients, after controlling for the number of exacerbations. Likewise, every exacerbation event increased the odds of antibiotic resistance by 41% (95% CI 6%, 88%) after controlling for hospitalisation status.

Analysis of H. influenzae samples

Typing of H. influenzae samples for the presence of capsular polysaccharides demonstrated that 29 out of 30 sputum isolates were nontypeable strains of H. influenzae and 1 was an encapsulated type b strain. Analysis of outer membrane proteins on 13 different NTHi isolates showed that they were all different strains.

Features of the different microbiologic groups

The three most common findings on sputum analysis were H. influenzae, P. aeruginosa and no pathogenic microorganisms. Patients on the basis of the initial sputum samples were divided into three different subsets (H. influenzae, P. aeruginosa and no pathogens) and patient characteristics (clinical, CT and spirometry features) were correlated with the three different subsets. The no pathogen group had the least severe clinical features, less extensive disease and best lung function. The Pseudomonas group had the worst clinical features and lung function and the most extensive disease. Results are summarised in Table 5.

Discussion

This study described the microbiologic profile of a series of subjects with bronchiectasis followed up for an average period of over 5.7 years. The spectrum of bacteria isolated was similar to previous studies with H. influenzae being the most commonly isolated bacterium, followed by other common pathogens including Streptococcus pneumoniae, M. catarrhalis and P. aeruginosa.

Recent studies have assessed the microbiologic profile in bronchiectasis and the frequency of isolates often varies significantly between different locations. The two main pathogens isolated have been H. influenzae (mean of 42% and a range of 29–70%) and P. aeruginosa (mean of 18% and range of 12–33%). The behaviour of these two pathogens has

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**Table 4** Comparison between characteristics of patients with antibiotic resistant and sensitive pathogens.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Resistant (n = 27)</th>
<th>Sensitive (n = 62)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>58 ± 14</td>
<td>57 ± 14</td>
<td>0.598</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>21 (78%)</td>
<td>41 (66%)</td>
<td>0.324</td>
</tr>
<tr>
<td>Volume, (ml)</td>
<td>48 ± 30</td>
<td>41 ± 30</td>
<td>0.303</td>
</tr>
<tr>
<td>Hospitalization in past year, n (%)</td>
<td>15 (52%)</td>
<td>13 (21%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Exacerbations in past year, n (%)</td>
<td>4.0 ± 1.8</td>
<td>2.8 ± 1.7</td>
<td>0.007</td>
</tr>
<tr>
<td>Number of lobes with bronchiectasis on HRCT</td>
<td>2.5 ± 0.9</td>
<td>2.4 ± 1.0</td>
<td>0.930</td>
</tr>
<tr>
<td>FEV1 (% predicted)</td>
<td>69 ± 24</td>
<td>72 ± 26</td>
<td>0.590</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>84 ± 22</td>
<td>86 ± 24</td>
<td>0.688</td>
</tr>
</tbody>
</table>

Table shows the patient characteristics on initial assessment and the association between the presence/absence of antibiotic resistance on follow-up.

**Table 5** Features of different microbiologic groups.

<table>
<thead>
<tr>
<th></th>
<th>No pathogens (n = 19)</th>
<th>Haemophilus influenzae (n = 43)</th>
<th>Pseudomonas aeruginosa (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum volume (ml)</td>
<td>35 ± 22</td>
<td>42 ± 32</td>
<td>76 ± 32</td>
</tr>
<tr>
<td>Hospitalizations (past year)</td>
<td>2 (11%)</td>
<td>4 (13%)</td>
<td>7 (64%)</td>
</tr>
<tr>
<td>MRC dyspnoea score</td>
<td>1.8 ± 0.8</td>
<td>2.0 ± 0.9</td>
<td>3.1 ± 1.6</td>
</tr>
<tr>
<td>Exacerbations (past year)</td>
<td>2.1 ± 1.2</td>
<td>3.5 ± 1.8</td>
<td>4.1 ± 1.7</td>
</tr>
<tr>
<td>CT involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobes with bronchiectasis</td>
<td>2.2 ± 1.0</td>
<td>2.3 ± 0.8</td>
<td>2.9 ± 1.2</td>
</tr>
<tr>
<td>Spirometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1 (% predicted)</td>
<td>76 ± 33</td>
<td>71 ± 20</td>
<td>63 ± 30</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>88 ± 29</td>
<td>89 ± 19</td>
<td>78 ± 28</td>
</tr>
</tbody>
</table>

MRC = Medical Research Council.
not been well characterized in bronchiectasis but in other forms of chronic bronchial disease such as cystic fibrosis and COPD both have been shown to have many different strains.\cite{15,16} It has been recognised recently that both are capable of forming biofilms.\cite{17,18} There was a low incidence of *Staphylococcus aureus* isolates in this cohort and there is some evidence to suggest this pathogen is more prevalent in cystic fibrosis.\cite{19} Recent literature has emphasised the role of nontuberculosis mycobacterial (NTM) infection in bronchiectasis.\cite{20} Wickremasinghe et al.\cite{21} found that 2% of a United Kingdom cohort of subjects with bronchiectasis had NTM, the same result as the current study. One authority has suggested that NTM infection in the United States may be associated with the relatively low hot-water temperature.\cite{22}

As has been the case in previous studies a large proportion of subjects in this study did not have pathogenic bacteria isolated from purulent sputum. There are some factors that need to be considered to explain these findings. The specimens were obtained from standard sputum samples and other sputum collection methods may have higher yields. The gold standard for the collection of specimens remains the protected bronchoscopy method. A recent study showed that there was good correlation between bronchoscopic procedures and standard sputum culture in patients with bronchiectasis, (the yield of pathogenic bacteria from sputum was 52%, 61% from protected specimen brush and 56% from bronchoalveolar lavage).\cite{23} In subjects with negative cultures, pathogenic bacteria may only be found in low numbers and as a consequence not be isolated using standard cultures. The use of other techniques such as polymerase chain reaction may increase the yield. Some patients may have had infections with viruses, which have not been assessed in this study. It has been suggested that up to a third of exacerbations of COPD are due to viral infections.\cite{24} The role of viruses in bronchiectasis is not well documented.

There do not appear to have been any previous studies with long-term follow-up data on the sputum microbiology of bronchiectasis. Over 50% of patients had the same pathogen on follow-up review as on initial assessment. On follow-up there was a higher incidence of *P. aeruginosa* but the most common pathogen remained *H. influenzae*. Pasteur et al.\cite{12} studied a cohort of 150 adults with bronchiectasis and as part of their assessment all subjects had multiple sputum samples analysed over a 1 year period. This study found that 66% of subjects were colonized with bacteria. These two studies suggest that a large number of patients become colonized with one pathogen and previous sputum microbiology analysis may be useful in guiding antibiotic selection in bronchiectasis.

The incidence of antibiotic resistance increased between the initial assessment and follow-up review, particularly in *H. influenzae* and *P. aeruginosa* isolates. The frequency of exacerbations on initial assessment was strongly associated with the presence of antibiotic resistance on follow-up and this presumably arose from the higher use of antibiotics. Antibiotics were given as a course of 10 days with single drug use in over 80% of cases. As such the regimen used was fairly conservative and less than 15% of patients received long-term antibiotic therapy (defined as more than 1 month of continuous antibiotics). Previous hospitalisation was also a major factor associated with antibiotic resistance. These findings emphasise that more attention may be needed to prevent the development of antibiotic resistance in such patients. Potential strategies may include further reduction in antibiotic usage, use of multiple antibiotics simultaneously to prevent resistance and avoidance of hospital admission with more emphasis on outpatient parental antibiotic use.

Despite the fact that *H. influenzae* is the most commonly isolated pathogen in bronchiectasis there has been little analysis of its characteristics in this condition. This study confirmed that in bronchiectasis this bacterium is the nontypeable form (NTHi) similar to other forms of bronchitis.\cite{25} NTHi is a heterogeneous pathogen with over many different strains\cite{26} and there is considerable turnover with new strains being acquired periodically.\cite{27} It has been shown that more than half of a cohort of subjects attending a cystic fibrosis clinic in Melbourne had the same (epidemic) strain of *P. aeruginosa* that appeared to arise as a consequence of cross-infection.\cite{28} The findings of 13 different types in 13 different patients studied, makes it unlikely that this cohort has an epidemic strain of NTHi.

This cohort could be divided into 3 separate groups based on results from sputum; no pathogenic microorganisms, *H. influenzae* and *P. aeruginosa*. There was a spectrum of severity in these 3 groups. The subjects in this study form part of a cohort who had been previously studied by the authors who found that the cohort had progressive disease with worse lung function and symptoms over an 8-year follow-up period. It is possible that there may be an evolution of disease from no pathogenic bacteria to *Haemophilus* to *Pseudomonas* infection in bronchiectasis. This appears to occur in CF.\cite{29} This study did not have a long enough follow-up period to make any clear conclusions.

In conclusion, the most common isolates from sputum analysis in this cohort of bronchiectasis subjects were *H. influenzae*, no growth and *P. aeruginosa*. Over half of this cohort was colonized with the same bacterium over a 5-year follow-up period. Antibiotic resistance increased over time and was associated with frequency of exacerbations and hospitalisation.

**Acknowledgements**

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**References**