



LETTERS

A molecular comparison of microbial communities in bronchiectasis and cystic fibrosis

To the Editor:

Chronic bacterial infections play an important role in disease progression in patients with bronchiectasis (BX) and cystic fibrosis (CF). Although only a few per cent of all bacteria can be cultured routinely in the laboratory [1], most bacteria can be identified through sequencing the variable regions of their 16S rRNA gene [2]. DNA studies in patients with CF revealed a more complex microbiota than was identified by standard culture [3, 4], leading us to test by sequencing whether BX may have a similarly complex polymicrobial state.

Approval was gained from the Brompton, Harefield and NHLI Research Ethics Committee and all subjects provided written consent. We collected sputa from 11 patients with BX (mean \pm SD age years 59.6 \pm 11.6) and 10 with CF (mean \pm SD age years 30.8 \pm 5.3). An aliquot of sputum was cultured following CF Trust microbiology guidelines [5]. Following lysis and DNA extraction we amplified a hyper-variable region of the 16S rRNA gene, and cloned, sequenced and analysed the products as described [6]. 96 clones were analysed for each patient. Sequences are stored as GenBank accession numbers JN212577–JN214344.

At the time of study, five of 11 BX patients were being treated with *i.v.* antibiotics for an acute exacerbation. Four patients were receiving treatment for chronic fungal pulmonary disease. Other antibiotics (oral or nebulised) were being administered long term, either as long-term anti-infective agents (such as nebulised colomycin or tobramycin) or as long-term anti-inflammatory immune modulators (azithromycin). Nine of the 10 CF patients were being treated for an acute exacerbation (eight with *i.v.* antibiotics and one with oral antibiotics).

A total of 1768 high quality 16S rRNA gene sequences were obtained from the sputum samples of all patients. No amplification of bacterial DNA was obtained for one sample, due to the presence of an unidentified inhibitor of PCR.

A total of 48 different operational taxonomic units (OTUs) (indicating distinct bacterial sequences) was observed in the combined BX and CF samples, consisting of 42 BX group OTUs and 18 CF OTUs. 12 (25%) OTUs were common to both groups (fig. 1). The 48 OTUs were assigned to a genus by a >98% identity match after a BLAST (Basic Local Alignment Search Tool) search of known bacterial 16S rRNA gene sequences using RDP Release 10, update 15 [7].

Pseudomonas aeruginosa was the most common organism detected by sequencing, being present in 81% of BX and 55% of CF patients. When *P. aeruginosa* was detected in a sample it was invariably the predominant OTU present (fig. 1). Strikingly, *P. aeruginosa* was detected by sequencing in five patients with negative cultures. We found more than one *Pseudomonas* spp.

OTU present in two patients, supporting the observation that it is common for CF and BX patients to be infected with multiple strains of *P. aeruginosa* [8]. The 16S rRNA gene cloning results did not discern between *P. aeruginosa* and its mucoid form.

In the absence of *P. aeruginosa* other organisms were predominant in the sputum samples, such as: *Proteus mirabilis* for patient BX4; *Achromobacter* sp. in patients BX7, BX10, BX15 and CF18; an unclassified Proteobacteria which grouped closely to *Haemophilus influenzae* and *Proteus mirabilis* in patient BX16; an unclassified Firmicute which grouped closely to *Granulicetalla* sp. and *Streptococcus* sp. in patient BX17; *Staphylococcus aureus* in patient CF2; *Ralstonia* sp. in patient CF21; and *Stenotrophomonas maltophilia* in patients CF22 and CF23. All of these organisms may be pathogens contributing to the disease process.

In only 20% of patients was there a complete agreement between bacteria detected by standard microbial culture and 16S rRNA gene sequencing. There was a partial match between 16S rRNA gene sequence identity and culture results in 35% of samples and no match in 45% of samples. This result is consistent with previous reports that 30% of purulent sputum samples from BX patients fail to culture with standard microbial techniques [9], and confirms that molecular methods can detect the significant presence of bacteria in culture negative patients [10]. Positive cultures were not accompanied by specific sequences in four patients, reflecting the limitations of small numbers of sequenced clones to identify low abundance organisms.

Amongst other common organisms, *Streptococcus* spp. were detected in 63% of BX patient samples and 30% of CF patient samples. *Prevotella* spp. were more common in BX (73%) compared to CF (22%). *Haemophilus* spp. were also present in 45% of BX and 10% of CF patients. In six patients more than one potentially pathogenic bacterium species was detected in >30% of the sample (fig. 1).

We did not estimate bacterial load, but all patients were producing purulent sputum at the time of the study. Inspection of the OTU distribution suggested a greater species diversity in BX patients compared to CF (fig. 1), which was confirmed by Chao estimates of the mean numbers of OTUs per sputum (8.2 \pm 5.2 in BX patients and 2.7 \pm 2 in patients with CF; $p < 0.0001$, LIBSHUFF estimate = 0.004). The microbiome from both sets of specimens was markedly different from that observed in the airways of control samples from adults and children [6]. The small sample and the use of multiple antibiotics made it difficult to dissect out whether the observed levels of diversity were related to acute and chronic antibiotic use, patient age or exacerbation status.

We have studied patients at a single time in the course of a prolonged illness. The technology we have used to obtain

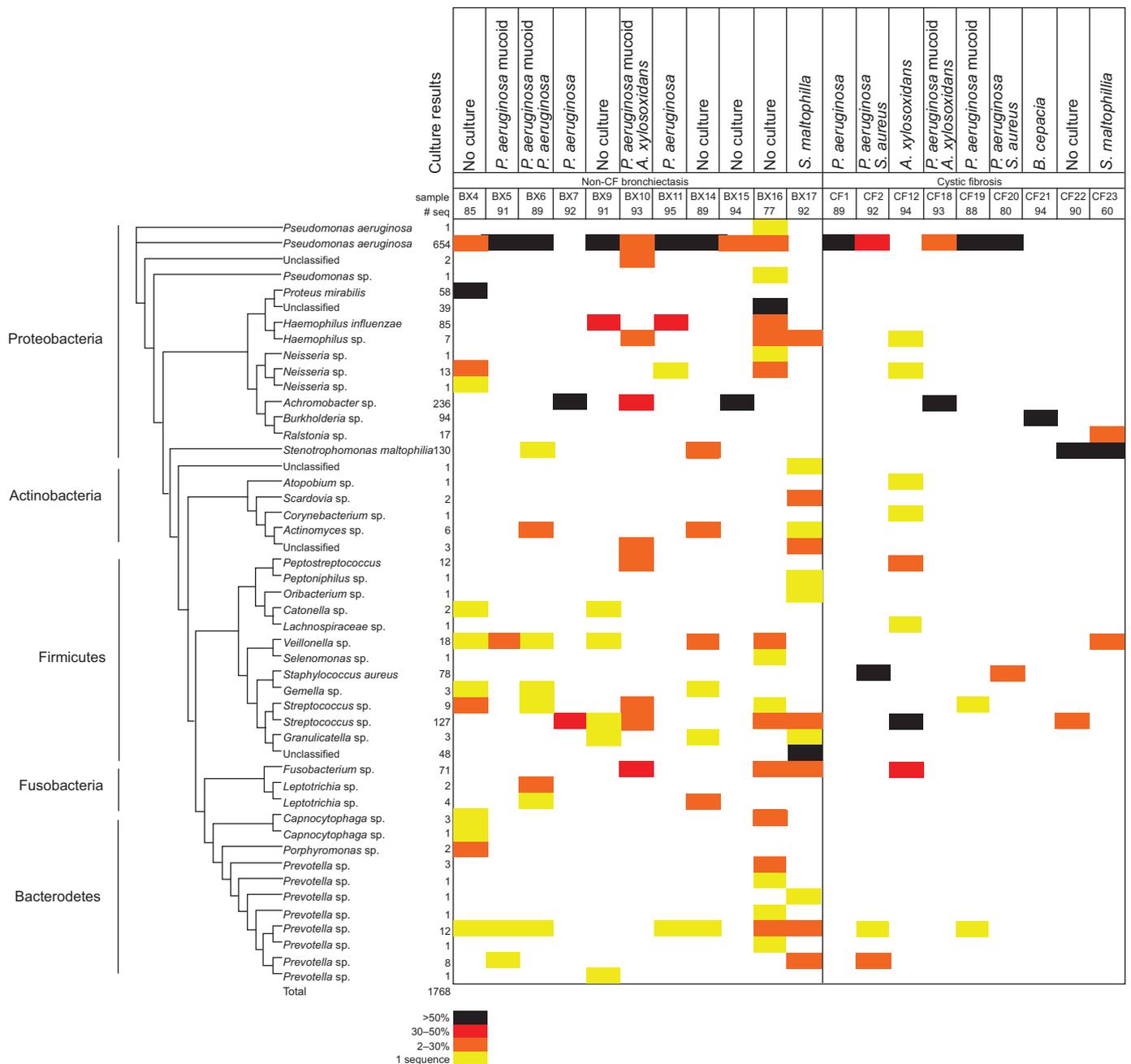


FIGURE 1. Heat map displaying the relative abundance of distinct bacterial species (defined as operational taxonomic units (OTUs)) detected by sequencing the 16S rRNA gene in sputum samples from each patient. The samples are divided into two panels showing results for non-cystic fibrosis bronchiectasis (BX) and cystic fibrosis (CF). The darkness of the bars indicates the relative abundance of each species in sputum samples from each patient. A phylogenetic tree showing the relationship between OTUs is aligned with the heat map to the left and culture results observed for each sputum sample are displayed at the top of the figure.

sequences (cloning and sequencing) is cumbersome and provides small numbers, and is already being replaced by rapid and potentially high-throughput ultra-deep sequencing of amplicons of the 16S rRNA gene. Our findings are nevertheless consistent with a similar study of resected lungs from patients with these illnesses [11]. Our findings show the limitations of sputum culture in identifying all bacteria that are causal in disease progression. Our results provide hope that the integration of molecular methods into the practice of clinical microbiology will result in an improved level of diagnostic accuracy.

Rachael M. Duff*, Nicholas J. Simmonds[#], Jane C. Davies^{#,†}, Robert Wilson⁺, Eric W. Alton^{#,†}, Panagiotis Pantelidis[†], Michael J. Cox*, William O.C.M. Cookson*, Diana Bilton^{#,5}, Miriam F. Moffatt^{*,5}

*Molecular Genetics and Genomics Section, National Heart and Lung Institute, Imperial College, London, [#]Dept of Cystic Fibrosis, Royal Brompton and Harefield NHS Foundation Trust, London, [†]Dept of Gene Therapy, National Heart and Lung Institute, Imperial College, London, and ⁺Host Defence Unit, Royal

Brompton and Harefield NHS Foundation Trust, London, UK.

⁵These two authors contributed equally to the study.

Correspondence: M.F. Moffatt, Molecular Genetics and Genomics Section, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, UK. E-mail: m.moffatt@imperial.ac.uk

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Exacerbation of respiratory symptoms in COPD patients may not be exacerbations of COPD

To the Editor:

Exacerbations of chronic obstructive pulmonary disease (COPD) are defined as acute events characterised by a worsening of the patient's respiratory symptoms, particularly dyspnoea, beyond day-to-day variation, leading to a change in medical treatment and/or hospitalisation [1, 2]. Exacerbations of COPD are a leading cause of hospitalisation and healthcare expenditures, particularly in frail, elderly patients. They alter the health-related quality of life and the natural course of disease, increasing the risk of mortality, both during and after the acute event [1, 2]. Patients with COPD frequently have chronic comorbidities [1]. Several of these comorbidities may produce acute events, contributing to the increased morbidity and mortality in COPD exacerbations: acute myocardial infarction, congestive heart failure, cerebrovascular disease, cardiac arrhythmias and pulmonary circulation disorders [1].

By definition, acute exacerbations of COPD are considered respiratory diseases, with specific reference to the respiratory symptoms and to the organs involved (airways and lung). Indeed, respiratory viral or bacterial infections and air pollution are assumed to be the main causes of COPD exacerbations, but

the exact contribution of infections is difficult to establish, and the aetiology of a large proportion of exacerbations remains undetermined [1, 3, 4].

Although it is known that bronchoconstriction and hyperinflation contribute to the increase in dyspnoea in COPD patients, the chronic airway, pulmonary and systemic inflammation present in patients with stable COPD is associated with an acute transient inflammatory process when respiratory symptoms are exacerbated by infections and/or pollutants. Neutrophils and/or eosinophils increase in the airways and lung, together with inflammatory mediators and protein leakage (fig. 1). This acute "respiratory" inflammation is associated with systemic inflammation, as shown by the increase in circulating inflammatory cells and pro-inflammatory cytokines. These systemic effects represent an important pathogenic link between COPD and comorbidities, particularly cardiovascular diseases [3, 4]. Indeed, epidemics of influenza are associated with an increased risk of death, particularly in elderly patients with chronic diseases, and the cause of death may be both respiratory and cardiovascular, particularly in the case of myocardial infarctions and stroke [5].