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ORIGINAL ARTICLE

Predominant pathogen competition and core microbiota divergence in chronic airway infection

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Chronic bacterial lung infections associated with non-cystic fibrosis bronchiectasis represent a substantial and growing health-care burden. Where *Pseudomonas aeruginosa* is the numerically dominant species within these infections, prognosis is significantly worse. However, in many individuals, *Haemophilus influenzae* predominates, a scenario associated with less severe disease. The mechanisms that determine which pathogen is most abundant are not known. We hypothesised that the distribution of *H. influenzae* and *P. aeruginosa* would be consistent with strong interspecific competition effects. Further, we hypothesised that where *P. aeruginosa* is predominant, it is associated with a distinct 'accessory microbiota' that reflects a significant interaction between this pathogen and the wider bacterial community. To test these hypotheses, we analysed 16S rRNA gene pyrosequencing data generated previously from 60 adult bronchiectasis patients, whose airway microbiota was dominated by either *P. aeruginosa* or *H. influenzae.* The relative abundances of the two dominant species in their respective groups were not significantly different, and when present in the opposite pathogen group the two species were found to be in very low abundance, if at all. These findings are consistent with strong competition effects, moving towards competitive exclusion. Ordination analysis indicated that the distribution of the core microbiota associated with each pathogen, readjusted after removal of the dominant species, was significantly divergent (analysis of similarity (ANOSIM), $R=0.07$, $P=0.019$). Taken together, these findings suggest that both interspecific competition and also direct and/or indirect interactions between the predominant species and the wider bacterial community may contribute to the predominance of *P. aeruginosa* in a subset of bronchiectasis lung infections.
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Introduction

The World Health Organization has reported that the global burden of diseases is shifting from communicable to non-communicable diseases, with chronic conditions such as heart disease, strokes and lung diseases now being the chief causes of morbidity and mortality (Lopez *et al.*, 2006). Among the great challenges in studying the causes and treatment of chronic lung infections, is a consequence of Koch's postulates and the subsequent concept of infection pathogenesis summarised by the expression 'one microbe, one disease' (Nelson *et al.*, 2013). Koch's postulates have shaped our understanding of medical microbiology, as many important microbial diseases conform to them. However, that orthodoxy is being undermined by a growing recognition that diverse important diseases, including chronic lung infections, have a polymicrobial aetiology. This expanding understanding of chronic polymicrobial infections, originating from studies of cystic fibrosis (CF) airway microbiota, is beginning to be translated to other chronic lower respiratory diseases, including non-CF bronchiectasis (hereafter referred to as bronchiectasis).

Bronchiectasis is a chronic airway disease characterised by abnormal destruction and dilation of the large airways, bronchi and bronchioles (Cohen and Sahn, 1999). It is associated with chronic and frequently purulent expectoration, multiple exacerbations and progressive dyspnoea that can become disabling (Ellis *et al.*, 1981; Cohen and Sahn, 1999; Barker, 2002), and represents a substantial and growing health-care burden. A recent US study demonstrated a marked increased prevalence in older populations varying from 4.2/100 000 adults aged 18–34 years to 271.8/100 000 aged $\geqslant$75 years (Weycker *et al.*, 2005). Bronchiectasis often goes unrecognised or is misdiagnosed as asthma or

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chronic obstructive pulmonary disease, leading to an underestimated prevalence. Despite this, bronchiectasis is associated with substantial socioeconomic cost due to the frequent use of primary and secondary health-care resources. An US epidemiological study of bronchiectasis-associated hospitalizations from 1993 to 2006 demonstrated an average annual hospitalization rate of 16.5/100 000 population with a significant annual increase of 2.4% in men and 3% in women (Seitz *et al.*, 2010), with the cost of managing bronchiectasis appearing to be rising (Joish *et al.*, 2013).

Airway inflammation resulting from chronic bacterial infection is thought to be a significant contributory factor driving disease progression in bronchiectasis (Barker, 2002). The perceived importance of bacterial pathogens in airway disease progression is reflected in the use of antibiotics (Serisier and Martin, 2011; Serisier *et al.*, 2013a), both as maintenance therapy and to treat episodes of acute exacerbation. However, our understanding of the mechanisms that underpin relationships between infection by particular bacterial taxa and clinical outcomes is currently poor. This situation undermines the development of rationales for the selection of particular antibiotic treatment regimes (Serisier, 2012) or potentially specific anti-inflammatory therapy (Visser *et al.*, 2012), and achieving better insight into the manner in which treatments achieve beneficial outcomes. Whereas bronchiectasis can result from a variety of recognised aetiologies, it is often considered idiopathic. Recent studies have revealed a substantial and diverse bacterial microbiota (Tunney *et al.*, 2013; Rogers *et al.*, 2013b, 2014; van der Gast *et al.*, 2014), which are typically dominated by either *Haemophilus influenzae* or *Pseudomonas aeruginosa*. Perhaps unsurprisingly given its colonisation of the upper airways in healthy individuals, *H. influenzae* is detectable in lower airway secretions from almost all bronchiectasis patients and is commonly the numerically dominant species (Rogers *et al.*, 2013b, 2014). In contrast, *P. aeruginosa*-dominated infections occur in a smaller number of patients (Rogers *et al.*, 2014) but are associated with an accelerated decline in lung function, more frequent pulmonary exacerbations, greater sputum production and a higher requirement for antibiotic therapy (Evans *et al.*, 1996; Ho *et al.*, 1998; Shoemark *et al.*, 2007; Rogers *et al.*, 2014).

A better understanding of the way in which *P. aeruginosa* interacts with the airway environment could provide important mechanistic insights into chronic infection in this patient group and in chronic respiratory infections more widely. Both the physiochemical characteristics of the airway environment and the composition of the pre-existing lung microbiota are likely to influence the likelihood of *P. aeruginosa* infection (Rogers *et al.*, 2013a). Further, where *P. aeruginosa* dominates the infection microbiota, its growth is likely to further affect the composition of airway environment. This impact of colonisation could occur both directly through the metabolomic (Kozlowska *et al.*, 2013) and secretomic (Bergamini *et al.*, 2012) footprint of *P. aeruginosa*, and, in turn, indirectly by stimulating changes in the host immune response (Bergamini *et al.*, 2012) and the activity of other co-colonising species (Bakkal *et al.*, 2010; Tashiro *et al.*, 2013). Whereas the causality in these interactions is difficult to demonstrate, were such relationships to exist, they would result in both an association between *P. aeruginosa* infection and measures of airway disease, and an association between *P. aeruginosa* infection and microbiota composition. The first of these associations has been well documented. However, to our knowledge, there have been no investigations to assess the second.

We hypothesised that (1) the distribution of *H. influenzae* and *P. aeruginosa* in airway samples would be consistent with strong interspecific competition effects; that is, when *H. influenzae* is the dominant species in a bronchiectasis lung infection, the population size of *P. aeruginosa* will be negatively influenced and *vice versa* when *P. aeruginosa* is dominant. (2) Where *P. aeruginosa* or *H. influenzae* is dominant species in a bronchiectasis lung infection, they are associated with distinct 'accessory microbiota' that reflect a significant interaction between these pathogens and the wider bacterial community. To test these hypotheses, we analysed 16S rRNA gene pyrosequencing data, generated previously, from 60 adult bronchiectasis patients whose airway microbiota was dominated by either *P. aeruginosa* or *H. influenzae*. *H. influenzae*-dominated infections were chosen as a comparator group as they had been shown previously not to differ significantly in total bacterial load, dominant taxon relative abundance or prior antibiotic burden (intravenous, oral and combined), with those where *P. aeruginosa* was dominant (Rogers *et al.*, 2014). Further, despite differences in disease course, these patients did not differ significantly in serum C-reactive protein levels, or sputum interleukin-8 and interleukin-1β levels (Rogers *et al.*, 2014) common markers of systemic and airway inflammation. To limit the potential effect of antibiotic therapy to influence microbiota composition (Serisier, 2013), a sample set was chosen where there had been a 4-week period of clinical stability before collection, with no supplemental antibiotics administered (Serisier *et al.*, 2013b).

Materials and methods

The analysis performed here was based on 16S ribosomal RNA gene pyrosequencing data generated from induced sputum samples from adult patients with bronchiectasis, as part of the BLESS trial (Serisier *et al.*, 2013b). These data are available through the Sequence Read Archive

(http://www.ncbi.nlm.nih.gov/sra) under the accession number SRP0356. Details of patient recruitment, sample collection, nucleic acid extraction, PCR amplification pyrosequencing and bioinformatics processing have been published previously (Rogers *et al*., 2013b; Serisier *et al*., 2013b; Rogers *et al*., 2014) and details of these processes are provided here as Supplementary Methods.

Of the 96 samples previously analysed, 26 were *P. aeruginosa*-dominated and 34 were *H. influenzae*-dominated. It is these 60 samples on which the analysis presented here is based. Patient details for these patients are shown in Table 1. As described previously, the species-level identification of *P. aeruginosa* and *H. influenzae* was confirmed using specific PCR-based assays in all instances (Supplementary Methods), with identification by pyrosequencing treated as presumptive. Where species-level identities were not corroborated by specific assays, identities are presented at the genus level.

Bacterial taxa within each metacommunity were partitioned into core and satellite groups using the Poisson distribution test as previously described (van der Gast *et al*., 2011; Rogers *et al*., 2013c). One-way analysis of variance, regression analysis, coefficients of determination (r^2), and residuals and significance (P) were calculated using the Minitab software (version 16, Minitab, University Park, PA, USA) as described previously (van der Gast *et al*., 2011; Rogers *et al*., 2013c). Canonical correspondence analysis, analysis of similarity (ANOSIM) and similarity of percentages analysis were performed using the PAST (Palaeontological Statistics, version 2.17) program available from the University of Oslo website link (http://folk.uio.no/ohammer/ past) run by Øyvind Hammer. Mann–Whitney tests were performed using Prism (version 5.01, GraphPad, La Jolla, CA, USA). Where predominant taxa were removed before analysis, the remaining relative abundance measures were rescaled and expressed as percentages.

Results and discussion

P. aeruginosa and *H. influenzae* share certain similarities, for example, they are both Gram negative, rod-shaped, facultative anaerobic Gammaproteobacteria. However, these common opportunistic pathogens are associated with very different clinical courses when dominant in bronchiectasis lung infections. Here the relative abundances of the two predominant species in their respective groups

Table 1 Clinical, treatment and comorbidity data for patient population

	P. aeruginosa		*H. influenzae*	
Gender (male:female)	6:20		16:18	
	Range	*Mean (± s.d.)*	*Range*	*Mean (± s.d.)*
Clinical measures of disease				
FEV1%*	30.7–94.2	60.5 (± 18.1)	37.2–114.7	71.2 (± 15.1)
Duration of bronchiectasis (years)	1–70	45.4 (± 19.6)	10–65	45.4 (± 17.5)
Pulmonary exacerbations in prior 12 months	2–12	6.3 (± 3.0)	2–7	3.4 (± 1.3)
Leicester cough score	5.5–20.2	14.8 (± 3.5)	7.7–19.9	15.6 (± 2.6)
SGRQ total	8.6–79.6	39.9 (± 16.6)	14.9–58.9	36.7 (± 14.0)
Six-minute walk test	291–650	488.7 (± 85.1)	275–710	519.6 (± 101.6)
C-reactive protein ($mg\,l^{-1}$)	0–21	6.8 (± 6.0)	0–19	7.1 (± 6.1)
Induced sputum IL-8 ($ng\,ml^{-1}$)	27.4–1053.8	337.6 (± 340.3)	28.8–1326.7	275.2 (± 295.7)
Induced sputum IL-1β ($ng\,ml^{-1}$)	0.25–36.2	9.8 (± 10.7)	0.6–115.8	11.4 (± 22.3)
Treatment				
Short acting β-agonist	14		10	
Inhaled corticosteroid	16		17	
Inhaled corticosteroid + long acting β-agonist	13		10	
Anti-cholinergics	6		2	
Aspirin	3		9	
Beta blocker	0		3	
Nasal steroids	2		4	
Prednisolone	3		1	
Antihypertensive	9		16	
Comorbidity				
Cerebrovascular disease	1		4	
Heart disease	2		4	
Hypertension	6		14	
Diabetes	1		0	

Abbreviations: FEV_1%*, forced expiratory volume in 1 s; SGRQ, St George's Respiratory Questionnaire.
FEV_1%*, expressed as a percentage of predicted and measured following administration of a bronchodilator. SGRQ, range 1–100, lower scores indicate better quality of life. Leicester cough score, lower scores indicate worse cough symptoms.

were high and not significantly different (analysis of variance, $F_{1,58} = 0.096$, $P = 0.758$; *P. aeruginosa* mean abundance and s.d. = 87.3 ± 13.4% and *H. influenzae* = 86.0 ± 17.9%; Figure 1). When present in the opposite dominated group, the two species were found to be in only very low abundances (*P. aeruginosa* = 0.37 ± 1.3% and *H. influenzae* = 0.56 ± 0.77%; analysis of variance, $F_{1,58} = 0.436$, $P = 0.511$). Furthermore, *P. aeruginosa* was not detected in 12 from 34 samples of the *H. influenzae* group, and 2 from 26 for *H. influenzae* in the *P. aeruginosa* group (Figure 1). This is consistent with strong competition effects between the two species, moving towards competitive exclusion of the inferior competitor species, and more so for *P. aeruginosa* within the *H. influenzae* group. If these patterns of dominance and suppression could be purely explained by the process of interspecies competition then no between-group differences in accessory microbiota would be expected. However, the analyses performed here demonstrate that taxa present in the microbiota associated with *P. aeruginosa* and *H. influenzae* predominance are significantly divergent.

The distribution of the two sets of microbiota, as determined by direct ordination using Bray–Curtis similarity measures, is shown in Figure 2. Where the dominant taxa (*P. aeruginosa* or *H. influenzae*) were included (Figure 2a), divergence in the distribution of the microbiota was pronounced and statistically significant (ANOSIM, $R = 1$, $P < 0.0001$). However, given the high proportion of total bacterial abundance that these predominant taxa would account for within the microbiota (Figure 1), much of the variation between the two groups will result from their inclusion in the analysis. In order to assess whether accessory microbiota composition differed significantly between the two groups, the predominant taxa were removed and the relative abundances of the remaining taxa redistributed and expressed as percentages. When subjected to ordination analysis, the distribution of the accessory microbiota composition (Figure 1b) was not found to be significantly divergent (ANOSM, $R = 0.036$, $P = 0.11$).

Many accessory microbiota taxa are of low relative abundance. The potential contribution to accessory microbiota of transient bacterial populations within the airways, as opposed to populations of chronically infective bacteria, is therefore high. In order to reduce the effect of these satellite taxa, the core microbiota (composed of non-randomly distributed taxa) in each of the two groups was determined (Figure 3). This is an approach that has been applied successfully in the analysis of chronic bacterial infections associated with CF (van der Gast *et al.*, 2011; Rogers *et al.*, 2013a). In the *Haemophilus*-dominated group, 9 of the 92 taxa detected were classified as core and 83 as satellite, and in the *Pseudomonas*-dominated group, 8 of the 70 taxa detected were classified as core and 62 as satellite. In each case, the contribution of individual taxa to the core microbiota was assessed using the similarity of percentages analysis (Table 2 and Supplementary Table S1). Ordination analysis was then performed using the core taxa, and again the difference between the distribution of the core microbiota that included the predominant *P. aeruginosa* and *H. influenzae* populations was significant (ANOSM, $R = 1$, $P < 0.0001$; Figure4a). Moreover, the divergence in the distribution of the core microbiota, readjusted after removal of the dominant species, was also found to be significantly divergent (ANOSIM, $R = 0.07$, $P = 0.019$; Figure 4b). Satellite taxa were not significantly different between the groups (ANOSIM, $R = 0.05$, $P = 0.06$).

To assess whether significant differences existed in the relative abundances of specific core taxa between the *P. aeruginosa*- and *H. influenzae*-dominated samples, Mann–Whitney tests were used and performed on readjusted core taxa abundance data after *P. aeruginosa* and *H. Influenzae* are removed. This process identified *Prevotella* spp. and *Flavobacterium* spp. as being significantly

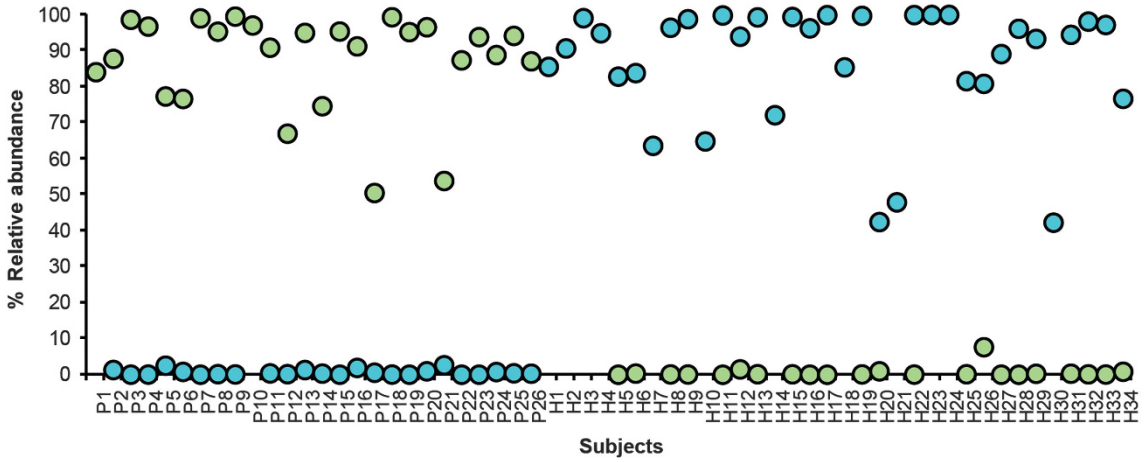

Figure 1 Relative percentage abundances of *P. aeruginosa* (green circles) and *H. influenzae* (blue) in samples from within *P. aeruginosa*- and *H. influenzae*-dominated groups (P1-P26 and H1-H34, respectively).

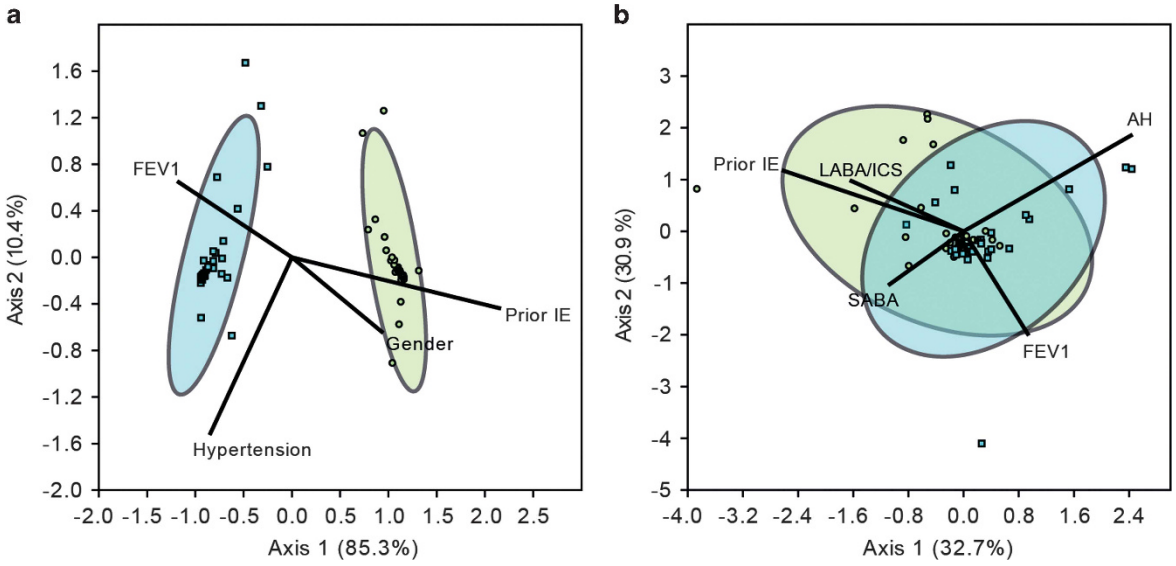

Figure 2 Canonical correspondence biplots for microbiota (**a**) with and (**b**) without *Pseudomonas* and *Haemophilus* included. Dots represent microbiota samples from the *Pseudomonas* (denoted with green filled circles) and *Haemophilus* (blue filled squares) groups. In each instance, the 95% concentration ellipses are given for the *Pseudomonas* (green) and *Haemophilus* (blue) groups. Biplot lines for the clinical variables included in the analyses show the direction of increase for each variable, and the length of each line indicates the degree of correlation with the ordination axes. CCA field labels: 'AH', antihypertensive; 'LABA/ICS', long acting β-agonist; 'SABA', short acting β-agonist; 'prior IE', number of pulmonary exacerbations in the prior 12 months. Percentage of community variation explained by each axis is given in parentheses.

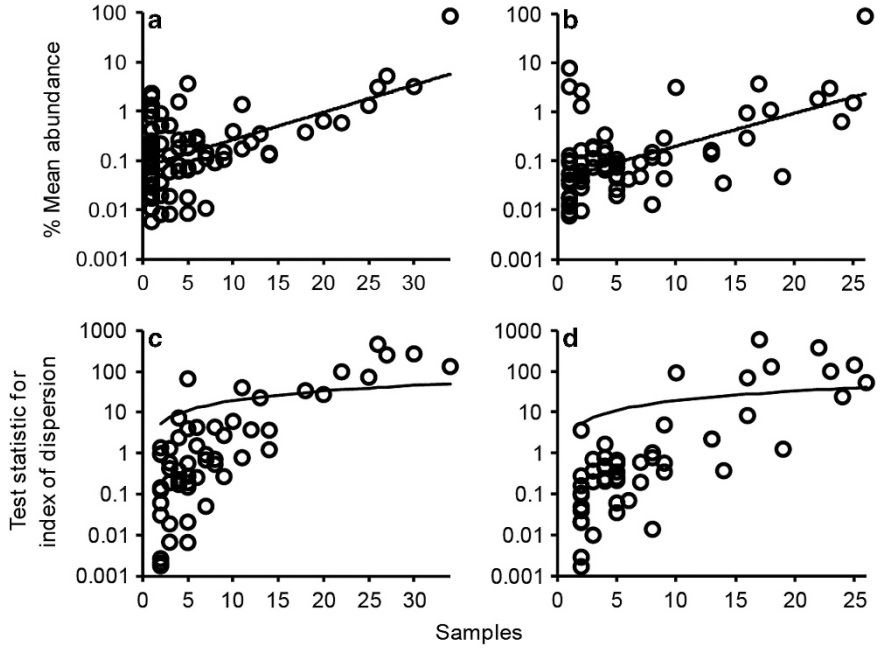

Figure 3 Distribution and dispersal of bacterial taxa among *Haemophilus*- and *Pseudomonas*-dominated microbiota samples. (**a** and **b**) The number of samples for which each detected bacterial taxon (open circles) was observed, plotted against the abundance ($\log_{10}$ scale) of that species among all samples within each group ((**a**) *Haemophilus* group, $r^2 = 0.27$, $F_{1,92} = 33.2$, $P < 0.0001$; and (**b**) *Pseudomonas* group, $r^2 = 0.33$, $F_{1,68} = 33.5$, $P < 0.0001$). In addition, given are dispersal plots to identify which bacterial taxa are randomly distributed within the (**c**) *Haemophilus* and (**d**) *Pseudomonas* groups; a measure used to assign core versus satellite status. Index of dispersion was calculated as the ratio of variance to the mean of abundance for each taxon within each group and plotted for each sample. The line depicts the 2.5% confidence limit for the χ^2 distribution. Taxa that fall below this line are randomly distributed and were considered satellite taxa, whereas those that are above the line are non-randomly distributed and were considered core taxa. The 97.5% confidence limit was not plotted, as no taxon fell below that line.

more abundant in the *P. aeruginosa*-dominated samples ($P < 0.0001$ and $P = 0.003$, respectively), whereas *Neisseria* spp. was significantly more abundant in *H. influenzae*-dominated samples ($P < 0.0001$). *Flavobacterium* is a genera that has been reported previously to contribute to bacterial

Table 2 Similarity of percentages (SIMPER) analysis of bacterial community dissimilarity (Bray–Curtis) between core taxa groups without *Pseudomonas* and *Haemophilus*

Taxon	*% Mean abundance*		*Samples detected in*		*Average dissimilarity*	*% Contribution*	*Cumulative %*
	Pseudomonas group	*Haemophilus group*	*Pseudomonas group*	*Haemophilus group*			
Prevotella	31.9	31	23	27	14.96	24.17	24.17
Veillonella	20.8	26.7	25	30	11.85	19.14	43.31
Streptococcus	13.2	18.3	22	26	10.51	16.99	60.30
Moraxella	14	4.9	10	5	8.69	14.04	74.34
Neisseria	7.8	13.6	18	25	7.59	12.26	86.60
Flavobacterium	8.7	0	17	0	4.35	7.02	93.62
Leptotrichia	3.6	2.0	16	18	2.22	3.59	97.21
Fusobacterium	0	3.5	0	11	1.72	2.79	100

Given is the mean % abundance of sequences for each taxon across the samples they were observed to occupy. Analysis was based on an average of 10 647 sequences per sample (s.d. 5070, range 2395–28 916). In addition, given is the average dissimilarity between samples (overall mean = 61.9%). Percentage contribution is the mean contribution divided by the mean dissimilarity across samples. SIMPER analysis with *Pseudomonas* and *Haemophilus* is presented in Supplementary Table S1.

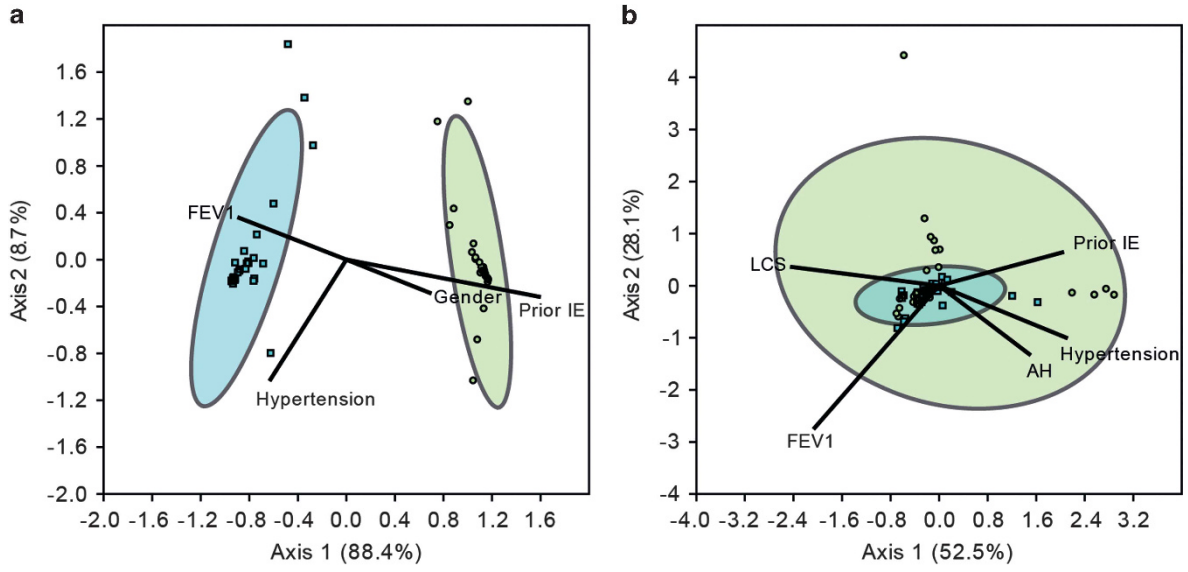

Figure 4 Canonical correspondence biplots for core microbiota (**a**) with and (**b**) without *Pseudomonas* and *Haemophilus* included. Dots represent core microbiota from the *Pseudomonas* (denoted with green filled circles) and *Haemophilus* (blue filled squares) groups. In each instance, the 95% concentration ellipses are given for the *Pseudomonas* (green) and *Haemophilus* (blue) groups. Biplot lines for the clinical variables included in the analyses show the direction of increase for each variable, and the length of each line indicates the degree of correlation with the ordination axes. CCA field labels: 'AH', antihypertensive; 'LCS', Leicester cough score; 'prior IE', number of pulmonary exacerbations in the prior 12 months. Percentage of community variation explained by each axis is given in parentheses.

communities present in chronic lung infections (Rogers *et al.*, 2003, 2004; van der Gast *et al.*, 2011; Rogers *et al.*, 2013a), although typically present at low relative abundances. In contrast, *Prevotella* spp. have been reported as both common and often at high abundance in both bronchiectasis and CF lung infections (Tunney *et al.*, 2008; Field *et al.*, 2010; Stressmann *et al.*, 2012) and otitis media (Brook, 2008). *Prevotella* spp. appear to be particularly prevalent when co-colonising with *P. aeruginosa*, a factor that has led to the previous suggestion that a synergistic relationship exists between *P. aeruginosa* and members of this genus (Su and Hassett, 2012). The genus *Prevotella* is composed of species that are obligate anaerobes. Whereas *P. aeruginosa* and *H. influenzae* are both capable of fermentation and growth under anaerobic conditions (Schobert and Jahn, 2010; Langereis and Hermans, 2013), the contributions of thick mucoid secretions that *P. aeruginosa* can produce in the airways (Ma *et al.*, 2012), a trait not demonstrated by *H. influenzae (*Langereis and Hermans, 2013), may contribute to reduced oxygen permeation, leading to the creation of greater opportunities for the growth of strict anaerobes, such as *Prevotella* spp. This model would be consistent with the association observed here between *H. influenzae* and *Neisseria*, a genus of typically aerobic species.

Canonical correspondence analysis was performed next to assess the extent to which variance in the microbiota distribution can be accounted for by variation in measures of disease severity, the presence of comorbidities and non-antibiotic therapies. The results of these analyses are shown in Table 3, and additionally superimposed on Figures 2 and 4. In keeping with previous reports (Rogers *et al.*, 2014), the presence of *P. aeruginosa* in samples as the predominant taxon was associated with high pulmonary exacerbation frequency and poor lung function (low forced expiratory volume in 1 s percent-predicted), with these factors varying significantly with microbiota distribution. However, whereas such clinical measures are associated with the presence of *P. aeruginosa*, these analyses also show that a significant relationship exists with the wider airway microbiota; a significant relationship was identified here between the variance in core taxa and Leicester Cough Score (a measure of cough symptom severity).

Here we observed distributions of predominant taxa consistent with strong interspecific competition, supporting competitive exclusion in some instances. However, we also observed, for instance, that where *P. aeruginosa* is the numerically dominant species in a bronchiectasis lung infection it is associated with a distinct accessory microbiota; suggesting in addition to interspecific competition, there are also direct and/or indirect interactions between the predominant species and the core microbiota. To some extent, such an effect was also observed for *H. influenzae* but was far less pronounced. However, several different models could explain such interactions. For example, the predominant species could influence the accessory microbiota composition through modification of the airway environment and alteration of its selective properties (and *vice versa*); here, perhaps the fact that *H. influenzae* is a common resident of the upper airways means that its presence is less disruptive to the commonly occurring infective lower airway microbiota. Alternatively, the same change in the characteristics of the airway could occur through intermediary interaction with the host that results in an altered inflammatory profile, airway secretion composition or secretion clearance rate. Here, a wide array of virulence factors and pro-inflammatory traits possessed by *P. aeruginosa* (Sadikot *et al.*, 2005) may contribute to the magnitude of the effect of its predominance on the accessory airway microbiota. Finally, external influences, such as antibiotic therapy, are likely to contribute to selective pressures in the airway environment. While there was no significant difference in historical antibiotic burden in the *H. influenzae*- and *P. aeruginosa*-dominated groups, the contribution of more subtle differences in treatment history cannot be excluded. We suggest that no single process is responsible for the associations observed, and rather a dynamic interaction between many different factors give rise to the various types of microbiological scenario seen *in vivo*. The potential complexity of these interactions makes their elucidation challenging. However, discerning their basis is important, given that a number of important clinical questions arise from the findings we present here. These questions include: (1) could accessory microbiota composition predict subsequent *P. aeruginosa* predominance and its associated poor prognosis? (2) Could intervention aimed at altering the characteristics of the airway environment, or the composition of the accessory microbiota, reduce the likelihood of *P. aeruginosa* infection and predominance? (3) What are the mechanisms to promote the dominance of *H. influenzae,* and as a consequence competitively exclude or suppress *P. aeruginosa*?

In each case, addressing these questions will require *in vitro* competition experiments between the two dominant bacterial species and members of the core microbiota that we have identified as having likely interactions with *P. aeruginosa* and *H. influenzae*. In addition, longitudinal sample sets that span both clinically and microbiologically important time periods will allow us to better understand the *in vivo* mechanisms that lead to

Table 3 Canonical correspondence analyses for determination of percent variation in lung microbiota from bronchiectasis subjects by clinical variables

Variable	*Whole microbiota*		*Whole microbiota without P and H*		*Core microbiota*		*Core microbiota without P and H*	
	% Of variance	*Probability*	*% Of variance*	*Probability*	*% Of variance*	*Probability*	*% Of variance*	*Probability*
Prior exacerbations	11.35	0.01	2.35	0.02	13.8	0.01	2.7	0.01
FEV_1% predicted	4.60	0.01	2.41	0.01	5.5	0.01	4.9	0.01
Hypertension	3.08	0.01	—	—	3.5	0.01	2.7	0.01
Gender	3.01	0.01	—	—	3.4	0.01	—	—
Antihypertensive	—	—	2.20	0.01	—	—	2.3	0.01
LABA + ICS	—	—	1.81	0.02	—	—	—	—
SABA	—	—	1.54	0.02	—	—	—	—
Leicester cough score	—	—	—	—	—	—	3.1	0.02

Abbreviations: FEV1, forced expiratory volume in 1 s; H, *Haemophilus*; ICS, inhaled corticosteroid; LABA, long acting β-agonist; SABA, short acting β-agonist; P, *Pseudomonas*.

predominance of *H. influenzae* or *P. aeruginosa* and its associated worse clinical outcomes. However, obtaining informative longitudinal sample sets in adult bronchiectasis has significant challenges. Intensive, long-term sample collection would be required to span rare and unpredictable events, such as the acquisition of *P. aeruginosa* in a condition that has relatively slow progression (Martínez-García *et al.*, 2007) and is commonly idiopathic (Anwar *et al.*, 2013). Given their potential to provide mechanistic insight into the relationship between recognised airway pathogens, the wider airway microbiota, host immunity and clinical outcome, such long-term frequent sample collection, represent an important next step.

In conclusion, we present evidence supporting the contribution of both interspecific competition and direct and/or indirect interactions between predominant infective taxa and the wider bacterial community, to determine whether *H. influenzae* or *P. aeruginosa* dominates the chronic lung infections associate with bronchiectasis. Given the prognostic implications of *P. aeruginosa* dominance, these findings provide a basis for identifying the mechanisms that underpin this airway microbial ecology, and perhaps offering novel therapeutic opportunities.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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