## 100 The airway microbiota in cystic fibrosis: a complex and dynamic biological community and implications for therapeutic management

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**Background:** Given the polymicrobial nature of pulmonary infections in patients with cystic fibrosis (CF), it is essential to enhance our knowledge on the composition of the microbial community to improve CF management. In this study, we developed a pyrosequencing approach to extensively explore the diversity and dynamics of fungal and prokaryotic populations in CF lower airways.
**Methodology and Principal findings:** Fungi and bacteria diversity in eight sputum samples isolated from CF patients was investigated using complementary conventional microbiological culturing and high-throughput pyrosequencing.
The unveiled microbial community structure was compared to the clinical profile of the CF patients. Pyrosequencing confirmed recently reported bacterial diversity and observed complex fungal communities, in which more than 60% of the species or genera had not been identified in cultures. Strikingly, the diversity and species richness of fungal and bacterial communities was significantly lower in patients with decreased lung function and poor clinical status. Phylogenetic analysis showed high molecular diversity at the sub-species level for the main fungal and bacterial taxa identified in the present study. Anaerobes were rarely isolated with *Pseudomonas aeruginosa*, which was more likely to be observed in association with *Candida albicans*.
**Conclusions:** In light of the new concept of microbiota, we viewed the microbial community as a unique pathogenic entity. We thus interpreted our results to highlight the potential interactions between microorganisms and the role of fungi in the context of improving survival in CF.

## 101 Characterization of the airway microbiota in the lungs of healthy individuals by strict anaerobic culture

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**Introduction:** Molecular based studies have recently challenged the view that the healthy lung is sterile and have shown that the lung may harbour its own unique microbiota. The aim of this study was to determine, using culture based methods, the composition of the airway microbiota in healthy controls age-matched to patients with both CF and non-CF related bronchiectasis (BE).
**Methods:** Induced sputum samples or cough swabs were collected from healthy volunteers and processed using strict anaerobic bacteriological techniques. Bacteria within the samples were detected by plating on selective agars, quantified by total viable count and identified by PCR and sequencing of 16S ribosomal RNA genes.
**Results:** Samples were collected from 29 healthy volunteers (n = 20 [9M:11F], age-matched to CF; n = 9 [6M:3F], age matched to BE) The mean (SD) age for volunteers in the age-matched CF and BE groups were 58.2 (4.7) and 30.4 (8.0) years, respectively. No bacteria were detected in samples from 2 CF controls with anaerobes not detected in a further 3 samples. Bacteria were detected in samples from all BE controls. Aerobes from a range of genera including *Streptococcus*, *Rothia* and *Staphylococcus* were detected in numbers ranging from $10^5$ to $10^7$cfu/g of sputum. Anaerobes from genera including *Prevotella, Veillonella,* and *Actinomyces* were also detected but in lower numbers ranging from $10^3$ to $10^6$ fu/g of sputum.
**Conclusion:** Aerobes and anaerobes have been detected by culture in the lungs of healthy people. Although the genera detected are similar to those we have previously detected in the lungs of patients with CF and BE, they are less frequently detected and present in much lower numbers.

## 102 Correlation between respiratory pathogen colonisation and lung function in a paediatric cystic fibrosis population

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**Objectives:** Our objective was to assess the cohort of patients attending our paediatric cystic fibrosis (CF) clinic for colonisation with 6 clinically significant pathogens and to determine if lung function differed between these groups.
**Methods:** We reviewed the results of all sputum samples over the previous 12 months on the cohort of paediatric CF patients attending our centre. We recorded colonisation with any of the following: *Staphylococcus aureus* (SA), methicillin-resistant *Staphylococcus aureus* (MRSA), *Stenotrophomonas maltophilia* (SM), *Acinetobacter species*, *Pseudomonas aeruginosa* (PA) [mucoid, non-mucoid or multi-drug resistant (MDRP)] and *Burkolderia cepacia complex* (BCC). We then correlated sputum results with mean forced expiratory volume in the first second percent predicted (FEV1).
**Conclusions:** Seventy of 90 paediatric patients attending our CF clinic were old enough to have reliable measurements of FEV1. Correlation of sputum culture reports with FEV1 was as follows: 38/70 (54.3%) colonised with SA with mean FEV1 of 85%, 7/70 (10%) colonised with MRSA with mean FEV1 of 67%, 23/70 (32.9%) colonised with PA with mean FEV1 of 75%, 6/70 (8.6%) colonised with SM with mean FEV1 of 81%, 3/70 (4.3%) colonised with *Acinetobacter species* with mean FEV1 of 87%, and 1/70 (1.4%) colonised with BCC with mean FEV1 of 76%.
Analysis of PA data revealed 13/23 (57%) colonised with non-mucoid PA (mean FEV1 86%), 10/23 (43%) colonised with mucoid ± non-mucoid PA (mean FEV1 70%) and no patient colonised with MDRP. In conclusion, CF patients with mucoid PA and MRSA constitute a small proportion of our total clinic cohort but have lower lung function and must be closely monitored.

## 103 Comparison of pharyngeal suction specimens and throat swabs in non-sputum-producing patients with cystic fibrosis

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To diagnose airway infection non-invasively is an important component of clinical care of CF patients. Sputum samples are used in older patients with more advanced disease with reliable results. Diagnostic problems may be significant in non-sputum producing patients.
We have compared 20 throat swabs and pharyngeal suction specimens taken contemporaneously in 18 CF patients (11M and 7F, mean age 9aa9m, range 2aa7m–27aa).
In 10 cases pharyngeal suction specimens culture was positive for bacteria species not identified in the throat swabs (4 *Pseudomonas aeruginosa*, 4 *Staphylococcus aureus* and 2 *Haemophilus influenzae*). In three cases the positivity for *P. aeruginosa* corresponded with the first isolation and had led to an eradication therapy. In two cases throat swabs were positive, for *P. aeruginosa* and *S. aureus* respectively, as pharyngeal suction specimens were negative. Pharyngeal suction, a procedure very low-time consuming and inexpensive, was well tolerated in all our patients.
Our results show that in non-sputum producing CF patients may be useful to associate pharyngeal suction to throat swabs for the diagnosis of lung infections. This more sensitive diagnostic strategy could above all anticipate the detection of first PA infection with great benefits.