100 Comparison of airway microbiota composition in healthy children and children with CF

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Introduction and Aims: Although a number of studies have characterized airway microbiota composition in healthy and CF adult populations, limited data is available for the paediatric population. The aim of this study was to define, using culture based methods, the composition of the airway microbiota in age-matched healthy and CF paediatric groups.
Methods: Induced sputum samples (CF) and cough swabs (CF, age-matched healthy volunteers) were collected and processed using both aerobic and strict anaerobic bacteriological culture techniques. Bacteria within the samples were detected by plating on selective agars and identified by PCR and sequencing of 16S ribosomal RNA genes.
Results: Samples were collected from 21 healthy volunteers (mean (SD) age 11.0 (3.5) yrs; 13M:8F) and from 12 children with CF (mean (SD) age 12.8 (3.2) yrs; 6M:6F) when clinically stable. Bacteria were detected in all samples from CF patients and in 18/21 (86%) healthy volunteers. Aerobic bacteria from genera including *Streptococcus, Staphylococcus, Pseudomonas* and *Haemophilus* were detected in both cohorts with *Pseudomonas* [CF, n = 2/12 (17%); control, n = 1/21 (5%)] and *Haemophilus* [CF, n = 7/12 (58%); control n = 4/21 (19%)] present in a greater number of CF patients compared to controls. Anaerobic bacteria from genera including *Prevotella, Veillonella,* and *Actinomyces* were detected in both cohorts.
Conclusion: Aerobic and anaerobic bacteria have been detected by culture in the lungs of children with CF and healthy children. *Pseudomonas* and *Haemophilus*, recognised as pathogens in CF patients, were more prevalent in CF patients compared to controls.
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101 Microbial communities and interactions in chronic lung infection in cystic fibrosis patients

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Bacteria in the natural environment form complex, interacting communities. This is also true for bacterial infections of animals and humans, where much of the diversity that we see may be driven by microbes interacting with each other as well as with the host. The Cystic Fibrosis (CF) lung facilitates the cohabitation of diverse microbial organisms and we are only just beginning to understand the extent of this diversity.
Objectives: Here, we aimed to study the community structure and interspecies interactions, particularly those affecting virulence, in greater detail by using both an in vitro model and deep sequencing of patient samples.
Methods: We have developed co-culture and multispecies biofilm models in which microbial interactions can be investigated. Using an artificial sputum medium, pathogens such as *Pseudomoas aeruginosa, Staphylococcus aureus, Burkholderia sp* and *Candida albicans* have been studied in a free-floating biofilm system to determine whether interspecies interactions facilitate the virulence of *P. aeruginosa*. To further characterise the CF lung microbiome, we have collected longitudinal sputum samples from adult CF patients and sequenced 16S rRNA sequences using an Illumina MiSeq. This enables the microbial community in each sample to be identified and compared to determine whether additonal factors influence the microbial composition of the lung over extended periods of time.
Conclusion: Considerable differences in the microbial population could be found between samples. Understanding these complex interactions may uncover novel therapeutic targets and ultimately lead to altered CF patient management.

102 Denitrification by cystic fibrosis pathogens

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Objectives: Chronic *Pseudomonas aeruginosa* lung infection is the most severe complication for cystic fibrosis (CF) patients. We have recently demonstrated on-going denitrification in sputum from patients infected with *P. aeruginosa*. Therefore we have speculated if the ability to grow anaerobically by denitrification relates to the known pathogenicity of CF pathogens.
Methods: We measured the growth by CFU/ml and OD_{600} in isolates from CF patients with chronic *P. aeruginosa*, *Achromobacter xylosoxidans*, *Burkholderia multivorans* and *Stenotrophomonas maltophilia* infections. All isolates were assayed after incubation in anaerobic LB with NO_3^- or NO_2^- for 2 days at 37ºC.
Results: With supplemental NO_3^- (10 mM) we found increased CFU/ml and OD_{600} for all pathogens. In *P. aeruginosa* and *A. xylosoxidans* we found higher OD_{600} than in *B. multivorans* and *S. maltophilia*. More CFU/ml was seen in *P. aeruginosa* than in *S. maltophilia*. With supplemental NO_2^- (10 mM) *P. aeruginosa* showed the highest CFU/ml and OD_{600}.
Conclusion: The anaerobic growth with supplemental NO_3^- was faster for the pathogenic *P. aeruginosa* and *A. xylosoxidans* than for the low-pathogenic *S. maltophilia* suggesing that reduction of NO_3^- contributes to the pathogenicity of *P. aeruginosa* and *A. xylosoxidans*. In addition, *P. aeruginosa* grew better with supplemental NO_2^-. The low growth by denitrification of *B. multivorans*, however, did not appear to relate to the its known high pathogenicity. Ongoing investigation of events further down-stream in denitrification is expected to further elucidate the involvement of denitrification in the pathogenicity of CF pathogens.

103 Bacterial diversity and dynamics during the early stages of *Pseudomonas aeruginosa* colonization in cystic fibrosis airways

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Objectives: To describe the bacterial diversity and dynamics of the cystic fibrosis (CF) airway microbiota during the period preceding *Pseudomonas aeruginosa* (PA) acquisition.
Methods: We characterized the bacterial communities of 17 iterative sputum samples from 5 CF patients including 1 adult and 4 children. These patients were PA-free for at least one year and underwent a longitudinal follow-up of 22 months in average. All patients became PA-positive in culture during the follow-up. PA was also investigated directly on sputa by quantitative RT-PCR targeting the *opr*L gene. Bacterial diversity was analyzed after amplification of the 16S rRNA gene and cloning/sequencing.
Conclusion: Out of the 1322 clones generated from the 17 sputa, 60.3% and 26.6% belonged to the Firmicutes and Proteobacteria phyla, respectively. Bacteroïdetes, Actinobacteria, Fusobacteria and TM7 phyla represented 7%, 4.8%, 0.6% and 0.07% of total clones, respectively. A total of 98 OTUs distributed in 34 genera were identified. On average, 27±7.4% of total clones were OTUs related to anaerobic bacteria. Persistence of anaerobic bacteria (such as *Prevotella* and *Veillonella* spp.) was observed in all sputa throughout the monitoring, even before PA colonization. A correspondence factor analysis revealed a relationship between the presence (or absence) of PA and the absence (or presence) of *Campylobacter* and *Haemophilus* spp.. In accordance with other studies, our results indicate that the composition of the lung microbiota may impact the early colonization of PA in CF patient. However, these hypotheses should be verified on a larger number of patients.