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WS8.1 The role of second-generation sequencing in describing the fungal microbiota in the adult cystic fibrosis (CF) airway and its correlation with clinical phenotype

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Objectives: The clinical role of fungus in the CF airway is uncertain. This study examines the fungal microbiota of the CF airway using a bar-coded high-throughput sequencing (HTS) method, and correlates with standard culture-based methods and clinical phenotype including FEV_1% predicted, genotype, gender, and BMI.
Methods: Sputa from 59 stable adult CF and 5 non-CF bronchiectasis patients were prospectively analysed.
Conclusion: Culture-based methods detected fungus (*Aspergillus* spp. and *Candida* spp. only) in 25% (n = 15/59) of patients. HTS with subsequent quantitative RT-PCR identified fungus in all samples, with a significantly greater abundance of fungus in the CF group (10^6 copies/ml) versus the non-CF controls (10^1 copies/ml). HTS revealed a complex fungal community in the stable CF airway with only 18% of species identified using culture-based methods. Correlating clinical phenotype with fungal microbiota diversity and species richness using multivariate linear regression revealed an inverse correlation between the abundance of *Malassezia* spp and lung function ($R^2 = 0.181$, $p = 0.04$). There was also a trend towards a positive relationship between the abundance of *Eleutheromyces* spp. and lung function ($p = 0.83$). No further association between the abundance of other fungal genera and clinical phenotype was seen. This study comprehensively examines fungal community dynamics in the CF airway. HTS methods highlight an unappreciated potential role of previously difficult to culture fungal strains like *Malassezia* spp within the CF airway. Understanding the clinical significance of fungal microbiota composition, diversity and richness may provide additional therapeutic targets.

WS8.2 Effect of *Staphylococcus aureus* on the outcome of pulmonary *Pseudomonas aeruginosa* infection in a murine model

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Previous studies have suggested that anti-staphylococcal therapy in young cystic fibrosis patients may favour early pulmonary colonisation with *P. aeruginosa*.
Objectives: This study examined the effect that colonisation of murine lungs with clinical isolates of *S. aureus* has on the outcome of *P. aeruginosa* infection.
Methods and Results: Groups of outbred mice (CD1) were either infected intranasally with a sub-lethal dose of various clinical isolates of *S. aureus* or given sham/PBS. Mice we allowed to recover and 12 days later were inoculated with *P. aeruginosa* intranasally. Mice which had administered Q154, a clinical isolate originating from the sputum of a chronically infected CF patient, had improved survival rates compared to mice which had been given Q181, an isolate which had previously caused toxic shock in a CF patient. Subsequent analysis of these two *S. aureus* strains revealed that they both had similar antibiotic susceptibility profiles and both were virulent strains in a moth larvae model of infection. Q154 produced protease *in vitro* whereas Q181 did not. Furthermore, Q154 also had an increased ability to produce biofilm *in vitro*. The lung cellular infiltrate and inflammatory cytokine profile have been characterised after infection with these two *S. aureus* isolates in order to gain further insight into the divergent impact of these two isolates.
Conclusions: Based on these results it is evident that *S. aureus* is able to modulate the outcome of *P. aeruginosa* infection in a murine model, with the impact being dependent on the *S. aureus* isolate. The mechanism for this diversity of influence remains to be elucidated.

WS8.3 Cystic fibrosis transmembrane conductance regulator (*CFTR*) allelic variants relate to shifts in fecal microbiota of cystic fibrosis patients

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Objectives: The human gastrointestinal tract is inhabited by a very diverse symbiotic microbiota, the composition of which depends on host genetics and the environment. The aim of the study was to investigate how *CFTR* gene variants could be related to fecal microbiota composition in cystic fibrosis (CF) patients. Around 1500 mutations and 300 polymorphisms of this gene are known, usually divided into five classes of severity.
Methods: *CFTR* genetic background was assessed in 36 CF patients, and fecal microbiota was investigated by TTGE fingerprinting technique and species-specific PCR. Multivariate statistical analyses were employed.
Conclusion: In an exploratory analysis we showed, through microbiota fingerprinting experiments, how severe CF patients withsevere *CFTR* mutations (e.g. F508del) harbor a different fecal microbiota structure than mild patients. We also showed, by species-specific PCR, how particular bacterial species are related to mild or severe clinical manifestations and to genetic background. Presence and relative abundance of *Eubacterium biforme* and *Escherichia coli* species were related to severe *CFTR* alleles, whilst *Eubacterium limosum* and *Fecalibacterium prausnitzii* species were related to the mild one. This is the first report that establishes a link between *CFTR* allelic variants and the corresponding shifts in fecal microbiota, opening the way to studies aimed at discovering possibly new bacterial markers in CF patients, depending on disease severity.

WS8.4 Temporal bacterial community dynamics of cystic fibrosis lung infections

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Objectives: Chronic lung infections are the biggest cause of morbidity and mortality of patients suffering from cystic fibrosis (CF). In recent years molecular based, cross-sectional studies of CF microbial ecology have identified a diverse and highly variable microbial community within the CF lung. Although these studies have provided useful insights into CF lung community ecology they have provided little information about community dynamics over time, during periods of both disease stability and exacerbation.
Methods: Fourteen patients attending the Southampton general hospital CF clinic provided sputum samples over the course of a year. DNA was extracted from these samples and analysed by directed 454 high-throughput pyrosequencing. Statistical analyses were carried out within an ecological framework to examine how the bacterial community within the CF lung changes over time, in the context of relevant clinical metadata.
Conclusion: We observed that during periods of stability the rate of bacterial species turnover (immigrations and extinctions) was also stable. However, leading into periods of clinical exacerbation, rates of turnover became high, mirroring worsening lung function. These changes were not confounded by intravenous (IV) antibiotic administration; where IV antibiotics are given once a patient is clinically judged to be in a period of exacerbation.
Our results suggest that the microbiota is changing before the introduction of IV antibiotics and these changes may serve as marker of disease severity with future implications for management of CF lung infections.