Objectives: DNA-based analyses of CF airway samples have challenged ideas about the etiology of CF infections. Two notable findings are: (1) In early CF lung disease, the microbial DNA in samples typically derives from diverse collections of microbes not considered conventional CF pathogens. (2) In established disease (when conventional CF pathogens dominate) non-conventional pathogens are still identified, although in lesser quantities. These findings are complicated because airway samples transit through the oropharynx where non-conventional pathogens are highly abundant. In addition, collection devices (e.g. bronchoscopes) and analysis reagents harbor microbial DNA.
Methods: We collected 190 bronchoalveolar lavage (BAL) samples from 22 children with CF after endotracheal tube intubation to attempt to bypass oropharyngeal organisms. We included controls to identify contamination from reagents, bronchoscopes, and the oropharynx. BAL was sequentially performed at 4–5 sites in each subject.
Results: BAL samples varied widely in the abundance of bacterial DNA (measured by qPCR). At sites with high levels of bacterial DNA, 16S rRNA gene sequencing showed that conventional CF pathogens dominated, and a relatively small fraction of microbial DNA mapped to non-conventional organisms. In contrast, sites with a low abundance of bacterial DNA contained DNA from a diverse collection of non-conventional organisms. Notably, the non-conventional organisms detected in most BALs were dissimilar from those found in the oropharynx, suggesting that intubation effectively bypassed upper airway contaminants. However, the non-conventional organisms in most BALs were highly similar to those found in control washes from bronchoscopes performed before the procedure.
Conclusion: Most of the non-conventional organisms in BALs were likely contaminants. The findings highlight the need for careful sampling and controls when DNA-based methods are used.

WS03.6
Estimation of total bacterial load in explanted cystic fibrosis (CF) lungs via qPCR

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Objectives: Regional variation in microbial load may contribute to variation in structural damage and disease progression in the CF lung. This study aimed to estimate total bacterial load in both tissue and sputum samples from distinct anatomical regions of explanted CF lungs using 16S rRNA quantitative PCR (qPCR).
Methods: Explanted lungs of CF patients were collected at transplantation, air inflated, frozen and cut into slices 2 cm thick. Cores (diameter = 1.4 cm) were removed from each slice and any sputum plugs within the tissue were excised. Following DNA extraction, the number of 16S copies/ml was quantified via probe-based absolute quantification using the LightCycler qPCR platform.
Results: Sixty-one tissue (n = 13 patients) and 11 sputum (n = 5 patients) samples were analysed. The number of 16S copies/ml was significantly higher ($p < 0.0001$, Mann-Whitney U test) in sputum compared to tissue. Furthermore, in 9 matched sputum and tissue samples (collected from n = 4 patients), the number of 16S copies/ml was also significantly higher ($p < 0.05$, Wilcoxon signed-rank test) in sputum compared to the surrounding tissue.
Nine patients had $n \geq 3$ cores analysed; in 8 of these patients, significant differences ($p < 0.05$, Kruskal-Wallis H test, Dunn's test *post-hoc*) in 16S copies/mL were apparent between different tissue cores.
Conclusion: The number of 16S copies/mL was significantly higher in sputum compared to tissue from explanted CF lungs. Significant differences in 16S copies/mL were also observed between different tissue cores from the same patient. This may contribute to regional variation in structural damage and disease progression observed in CF.
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WS03.7
Coexistence of prey and predator bacteria in cystic fibrosis lung microbiota

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Objectives: We aimed to monitor the lung microbiota composition of 15 CF-patients during 1-year follow-up in relation to their clinical data and antibiotic consumption.
Methods: Fifteen CF-adults contributed with 3–4 induced sputum samples during a follow-up period of one year. Samples were processed for conventional microbiology cultures and also submitted to massive sequencing by Hi-Seq Illumina platform to determine the lung microbiota composition. A new computational model based was designed to predict the ecological interactions between CF-pathogens and prey-predator bacteria over time. For this model we considered *P. aeruginosa, S. aureus* and *H. influenzae* as prey bacteria whereas the predator species were *Vampirovibrio* and *Bdellovibrio*.
Results: Microbiological cultures of the 56 sputum samples demonstrated chronic lung colonization by *P. aeruginosa* (11 patients), *S. aureus* (11 patients), *B. cepacia* (1 patient) and *Pandoraea* spp. (1 patient). *P. aeruginosa* and *S. aureus* co-colonization was observed in 8 patients with the lowest lung function. The Phyla distribution in the sequential sputum samples was not stable, detecting significant variations for *Proteobacteria*. Considering all samples, 156 bacterial species were detected, corresponding "90% to cultivable CF-pathogens. Unexpectedly, recognized predators as *Vampirovibrio* (17 samples, 12 patients, 0.003%) and *Bdellovibrio* (6 samples, 3 patients, 0.002%) were detected. Computational modeling results were consistent with the extinction of all populations except one predator and one prey that finally coexist. Finally, the introduction of a high initial predators population (0.15% instead 0.03%) predicted the extinction of both preys and predators
Conclusion: The presence of predator bacteria was described for the first time within the CF-lung microbiota. Computational modeling could help us to decipher bacterial ecology linked to CF-environment, and to propose novel decontamination interventions.

Highlights on exercise

WS04.1
The effect of Orkambi® on exercise capacity and muscle strength

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Objectives: CFTR expression plays an intrinsic role in skeletal muscle atrophy and dysfunction in Cystic Fibrosis (CF). Orkambi® is a new therapy for the treatment of CF with an action on the CFTR. It was made available to patients in Ireland with <40% predicted FEV1 in 2016. The aim of this study is to investigate changes in muscle strength and exercise tolerance in patients commencing Orkambi®. No previous study had been completed examining this relationship.
Methods: Subjects with CF and clinically stable disease were recruited on commencing Orkambi®. Baseline characteristics of age, BMI and FEV1 were recorded. Muscle strength was assessed by measuring isokinetic strength of quadriceps muscle on Biodex®, and grip strength by hand held dynamometry. Exercise capacity was measured using the 6 Minute Walk Test. No additional exercise intervention was prescribed following testing. Testing was repeated 3 and 6 months post commencing the drug.
Results: 17 subjects were recruited, 6 subjects were lost to follow up. 6 month follow up data was available for 9 subjects (however this is expected to rise to 11 prior to conference dates.) Repeat measure ANOVA was carried out on 6 Minute walk test distance, peak torque quads/body weight, quad acceleration time and grip strength. No significant differences were found in any of the four variables at 3 or 6 months.
Conclusion: The current study hypothesized that with the introduction of Orkambi®, muscle strength would improve without exercise intervention.