48 Interactions of microbes during chronic lung infection in cystic fibrosis patients

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Bacteria in chronic infections can form complex, interacting communities. These communities diversify as a result of interactions with both the environment and each other. The Cystic Fibrosis lung facilitates the cohabitation of diverse microbial organisms and we are only just beginning to understand the extent of this diversity.
Objective: 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 multispecies biofilm models in which microbial interactions can be investigated.
Results: 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*. In addition, metabolic footprinting of these cultures was used to determine changes in small-molecule metabolites. 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 additional factors influence the microbial composition of the lung. Considerable differences in the microbial population could be found between samples.
Conclusion: Understanding complex interactions may uncover novel therapeutic targets and ultimately lead to altered CF patient management.
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49 Subgingival plaque in patients with cystic fibrosis: potential source for airway colonisation

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Objectives: Subgingival plaque consists of both aerobic and anaerobic species which coexist to form a complex biofilm. The aim of this study was to determine (1) the bacterial composition of subgingival plaque collected from patients with CF and (2) if bacteria isolated from subgingival plaque are identical to those isolated from matched respiratory samples.
Methods: Subgingival plaque and sputum samples or cough swabs were collected from CF patients when stable or during periods of exacerbation and processed by aerobic and strict anaerobic culture. 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 processed from 10 CF patients (mean±SD age, 37±13.2 yrs; 8 female, 2 male; *P. aeruginosa* +ve n = 4, *P. aeruginosa* –ve, n = 6, as determined by routine laboratory culture). Bacteria normally associated with subgingival plaque, such as *Prevotella* sp., *Veillonella* sp. and *Streptococcus anginosus* were isolated from matched plaque and respiratory samples (n = 3). Matched isolates displayed sequence homology ranging from 99.9% to 100%. Surprisingly, *Pseudomonas* sp. was detected in the subgingival plaque of one patient +ve for *Pseudomonas* lung infection, with both isolates displaying the same morphotype.
Conclusion: *Pseudomonas* sp. in subgingival plaque may act as a reservoir for reinfection. Removal of subgingival plaque in this patient group may minimise potential reinfection from the oral cavity.
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50 Microbiological characterization of patients in Lisbon cystic fibrosis centre

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A persistent cycle of infection/inflammation happens in the lungs of cystic fibrosis patients. Microbial flora assumes a relevant role in pathologic process. The authors' aim is to characterize lung infection in different age groups of patients in Lisbon Cystic Fibrosis Centre.
Fifty patients were distributed in 4 groups: 0–5 (n = 10), 6–9 (n = 5), 10–14 (n = 20) and 15–18 (n = 15) years. The colonization of each pathogen was evaluated in 2013, and correlated with lung function (LF) and nutritional status.
Chronic colonization (CC) with *Staphylococcus aureus* (SA) was present in 48% of patients. Meticillin-resistant SA colonization was found in 10% of patients, none of them older than 15 years. *Pseudomonas aeruginosa* was present in all groups and 30% of patients had CC. *Burkholderia cepacia* was isolated in 20%, only in group 10–14, and was related with decrease in LF (FEV1 73.7% vs. 85.4% in non-infected). Intermittent colonization (IC) with *Haemophilus influenzae* occurred in 36%, and was isolated in all patients in 6–9 years group (60% with CC, 40% with IC). *Achromobacter xylosoxidans*, *Alcaligenes xylosoxidans* and *Stenotrophomonas maltophilia* were only found in patients older than 6 years, while *Enterobacter cloacae* was predominantly found in patients younger than 5. *Aspergillus* spp. colonization was found in 32%, predominantly in patients older than 15 (60%). Patients with this isolation had mean FEV1 86.7% vs. 92% without this colonization.
Age should be a factor to consider when antibiotics are prescribed, as differences in microbial flora were observed. SA was the main agent of CC and *Haemophilus influenzae* of IC. *Aspergillus* was frequent in older patients, but does not significantly affect LF.

51 The impact of coliforms isolated from cough swabs from infants under the age of one year with cystic fibrosis

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Objectives: To assess the clinical impact of isolating coliforms from cough swabs in infants less than one year of age with cystic fibrosis.
Methods: The notes of 82 children born after July 2004 and before September 2011 were reviewed. 26 children were excluded due to data availability or confounding variables. 49 children grew coliforms within the first year of life, 7 did not. Data was collected from birth to 18 months of age for nutritional parameters, GOR symptoms, exacerbations, pathogens grown and antibiotics required.
Results: Ten children grew coliform once, 27 intermittent, 12 chronic (as per *Pseudomonas* classification)1. 47% growers were treated for GOR versus 29% non-growers. Average SDS for HT and WT were lower at birth only in growers. The table illustrates infants who grew coliforms had more exacerbations, and were given more antibiotics than non-growers. An average of 1.4 other pathogens were identified per child in the growers group, versus 1.3-in the non-growers.

Table: Average number of exacerbations and antibiotics

	Antibiotics in 1st year				Antibiotics in 1st 18 months			
	Exacerbations	PO	IV	Neb	Exacerbations	PO	IV	Neb
Growers	5.82	5	0.54	0.06	8.37	7.51	0.76	0.31
Non-growers	5	3.57	0.29	0.29	7.67	5.5	0.5	0.33

Data are averages.
IV, intravenous; Neb, nebulised; PO, oral.

Conclusion: In the first year most children grow coliforms on cough swabs. There is a possible association between coliform growth and increased frequency of respiratory exacerbations, other pathogen growth, and requirement for antibiotics. There is no known evidence for pathogenicity of coliforms in infants with CF. Current practice is not to treat unless clinically indicated. Further studies are required to ascertain if this is the correct approach.