

of which have also been isolated from CF patients from other UK hospitals. Isolates from the remaining 3 patients were unique. One additional isolate was identified as *M. abscessus* subsp. *bolletii*. No clear epidemiological connexions between patients within each cluster at our centre have been identified to date.

Conclusion These results provide further evidence that some strains of *M. abscessus* complex may be isolated from multiple CF patients. However, there were no clear epidemiological connexions between patients within clusters at our centre. The same strains have been isolated from patients at different UK CF centres. Further studies are required to determine the mode of acquisition of infection with these strains and whether there is a common environmental source of infection or cross infection between patients.

P200 PRELIMINARY EVALUATION OF THE FUNGAL AIRWAY MICROBIOME IN ADULT CYSTIC FIBROSIS BY NEXT-GENERATION SEQUENCING, CULTURE AND STAINING TECHNIQUES

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Introduction The prevalence and diversity of fungal airway isolates is increasing in cystic fibrosis (CF). Amidst an extending spectrum of fungal complications, lack of standardised mycology methods and poor sensitivity of culture-dependent techniques renders interpretation of isolates challenging.

Aims To evaluate the diagnostic utility of fungal cytology and microbiology stains in addition to prolonged sputum culture from adult CF patients in comparison to standard mycology techniques.

Secondly, to develop a novel, next-generation sequencing assay targeting the ITS2 region of the fungal ribosomal-RNA gene to comprehensively profile the sputum fungal microbiota.

Methods Sputum samples were investigated by a panel of three mycology techniques: prolonged fungal culture (each examined at: Day7, D14, D21, D28); Calcofluor White (CFW) stain; Grocott's Methenamine Silver (GMS) stain. A cohort of samples was also subject to broad-spectrum fungal next-generation sequencing.

Results 25 adult patients provided 45 sputum samples. Four fungal species were cultivatable: *Candida* species (26.6%); *Aspergillus fumigatus* (4.4%); *Scedosporium apiospermum* (15.5%) and *Exophiala dermatitidis* (11.1%).

Prolonged culture significantly increased overall fungal prevalence by 22% compared to standard duration (D7) $p = 0.008$. A significant increase of 11.1% in *S. apiospermum* prevalence was observed $p = 0.02$, whilst all *E. dermatitidis* isolates required prolonged culture. The sensitivity of GMS and CFW stains (85% and 93%) compared favourably to standard duration culture (29%).

DNA extracted from a pilot group of these sputum samples ($n = 14/45$) was subject to PCR using barcode-indexed ITS2 primers designed for Illumina-MiSeq amplicon sequencing. Fungal taxa were detected in all samples, of which seven samples (50%) were negative after prolonged culture. Preliminary sequencing analysis of an extended sample cohort ($n = 30$) has detected 89 fungal taxa, from which only four species were cultured.

Conclusions Prolonged fungal culture is associated with a significant increase in fungal prevalence. The increased sensitivity is restricted to less common filamentous fungi associated with increasing pathogenicity: *S. apiospermum* and *E. dermatitidis*. The predictive value of stains in identifying samples positive at prolonged culture, but negative at standard duration illustrates their clinical utility.

Illumina-MiSeq ITS2-amplicon sequencing directly from sputum has identified a more diverse CF airways fungal microbiota. Preliminary analysis suggests that this is a highly sensitive tool for detecting fungi from sputum, including species which are refractory to standard and enhanced culture.

P201 PNEUMOCYSTIS JIROVECCII PREVALENCE IN A LARGE UK ADULT CYSTIC FIBROSIS CENTRE

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Introduction and objectives *Pneumocystis jirovecii* (PJ) is an atypical fungus that causes pneumonia in immunocompromised patients. Its role in patients with cystic fibrosis (CF) is unclear. Its reported prevalence in CF ranges from 1–22% but has never been determined in the UK. Here we present preliminary cross-sectional data from an ongoing study at a UK adult CF centre

Abstract P201 Table 1 Comparison of characteristics for patients with positive and negative samples

<i>Pneumocystis jirovecii</i> result			
	Negative	Positive	Sig.
Number	93	7	NS
Demographics			
Male gender, n (%)	52 (55.9)	4 (57.1)	NS
Mean age (yr), SD	33.8 ± 11.1	32.5 ± 10.1	NS
Spirometry and laboratory results			
Mean FEV ₁ (% predicted), SD	56 ± 20.1	67 ± 29.5	NS
Mean BMI (kg/m ²)	22.1 ± 3.2	22.2 ± 2.1	NS
WCC (x10 ⁹ /L) at recruitment	9.3 ± 3.1	9.3 ± 2.8	NS
CRP (mg/L) at recruitment	13.0 ± 16.8	7.9 ± 7.5	NS
Chronic colonisation, n (%)			
<i>Pseudomonas aeruginosa</i>	59 (63.4)	5 (71.4)	NS
<i>Staphylococcus aureus</i>	24 (25.8)	1 (14.3)	NS
<i>B. cepacia</i> complex	14 (10.3)	2 (28.6)	NS
<i>Achromobacter xylosoxidans</i>	6 (6.4)	0	NS
<i>Ralstonia</i> species	5 (5.4)	0	NS
NTM	5 (5.4)	1 (14.3)	NS
<i>Aspergillus fumigatus</i>	4 (4.3)	1 (14.3)	NS
<i>Pandora</i> species	1 (1.1)	1 (14.3)	NS
MRSA	1 (1.1)	1 (14.3)	NS
Treatment, n (%)			
Inhaled antibiotics	72 (77.4)	6 (85.7)	NS
Inhaled steroids	59 (63.4)	6 (85.7)	NS
Oral steroids (continuous)	12 (12.9)	1 (14.3)	NS
Prophylactic macrolide	77 (83.0)	5 (71.4)	NS
Azole therapy	8 (8.6)	0	NS
Co-trimoxazole past 3 months	32 (34.4)	0	0.06

Key: SD = standard deviation, WCC = serum white cell count, *B. cepacia* complex = *Burkholderia cepacia* complex, NTM = Non-tuberculous mycobacteria, MRSA = Methicillin resistant *Staphylococcus aureus*, NS = not significant, p values <0.1 are shown.