O468
2-hour Oral Session
Microbiome in disease and treatment

Comparison of culture with T-RFLP, 16S rDNA sequencing and metagenomics for routine diagnostics of respiratory microbiota in patients with cystic fibrosis

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Background: Cystic fibrosis (CF) is an autosomal recessive disorder. Mutations of the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene are associated with impaired mucociliary clearance and chronic respiratory infections. While culture is the current gold standard for routine diagnostics of respiratory infections in CF the culture-independent techniques are emerging tools for optimized diagnostics in upcoming years.

Material/methods: In the present multicentre study, a direct comparison of routine culture with 16S rDNA fingerprinting (terminal restriction-fragment length polymorphism, T-RFLP using HaeIII and HhaI), 16S rDNA sequencing (Roche 454) and metagenomics (SOLiD) was performed. Patients with CF in different age groups (A: 8-13 years, B: 18-23 years, C: >28 years) and clinically relevant subgroups (pancreas sufficient [PS] vs. pancreas insufficient [PI]) were included in two specialized centers (University of Saarland Medical Center, Homburg, and Hannover Medical School) and induced sputa of three consecutive visits were investigated after standardized nucleic acid isolation.

Results: The three culture-independent tests revealed qualitative and quantitative bacterial patterns each, although discrimination capacity was different. In each sample 2.91 ± 1.05 species were reported using routine culture. For T-RFLP 16.43 ± 7.24 (HaeIII) or 14.15 ± 5.31 (HhaI) peaks were found, 16S rDNA sequencing was assigned to 56.52 ± 19.11 operative taxonomic units (OTU), whereas metagenomics revealed 178.35 ± 185.97 bacterial species. The detection rates of particular CF pathogens were increased if culture-independent assays were applied; e.g. for Pseudomonas the

detection rate increased by 20% for 16S sequencing, 33% for T-RFLP (HaeIII) and 47% for metagenomics. Pathogens detected with high abundance were allocated mostly to cultivable and we could not identify as yet unrevealed bacteria predictive for respiratory disease in CF. Diversity indices associated with dominance (Simpson-index) or richness and evenness (Shannon-index) were similar for T-RFLP and 16S rDNA sequencing but lower for metagenomics according to higher richness detected by this method. Correlation coefficients were higher for metagenomics vs. 16S rDNA sequencing (R = 0.47; 0.43) and 16S rDNA sequencing vs. T-RFLP (HaeIII) (R = 0.49, 0.42) as compared to metagenomics vs. T-RFLP (HaeIII) (R = 0.35; 0.25). Global analysis of microbiota using non-metric multidimensional scaling (NMDS) was not different between age groups, clinical relevant subgroups (PS vs. PI) and also comparing patients with and without pulmonary exacerbations. However, culture-independent analysis proved to be a promising tool for standardized quantitative and qualitative analysis of respiratory microbiota in CF. Almost each patient was detected with an individual bacterial pattern which was described best using highly discriminative quantitative methods (metagenomics>16S rDNA>T-RFLP>culture).

Conclusions: Regular follow-up investigations using culture-independent analysis may be applicable soon for monitoring of patient´s microbiota. Changes in bacterial patterns may then be associated with disease progression or successful treatment resulting in reduction or elimination of CF-relevant bacteria.