Detection of microorganisms involved in airway infection of cystic fibrosis patients by standard culturing and molecular methods

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Patients suffering from the genetic disease cystic fibrosis (CF), develop chronic lung infection. This infection persists due to highly viscous mucus occurring as a result of the disease, in which bacteria form biofilms. Diagnostic tools for CF rely on culture based techniques performed on expectorated sputum samples, and most studies are centered on this sample type. It is however problematic to investigate biofilm-residing microorganisms, and further problems may occur since it is possible that the samples may be contaminated by oral flora during expectoration.

In this study tissue and sputum samples (n=24) from explanted lungs of four Danish CF patients were examined to circumvent possible oral flora contamination. Samples were examined by standard culturing techniques, including aerobic and anaerobic growth, at Rigshospitalet, Denmark. These findings were compared to results obtained by 16S rRNA gene analysis (16S rRNA gene amplification, cloning, sequencing and phylogenetic analysis) performed blinded of the growth results and quantification of the oprL gene of Pseudomonas aeruginosa by quantitative PCR at Aalborg University, Denmark.

The microorganism detected most often by 16S rRNA gene analysis in a sample was also detected by standard culturing techniques (which gave monomicrobial results). 16S rRNA gene analysis suggested that samples contained polymicrobial infection. A correspondence between the frequent detection of P. aeruginosa by the above methods and the number of P. aeruginosa cells in most samples were found by quantitative PCR. This suggests that detection of P. aeruginosa by culturing and 16S rRNA gene analysis is not caused by biases in the techniques.