

**104 Culture dependent and independent analysis of microbial community composition in the lungs of patients with cystic fibrosis and non-cystic fibrosis bronchiectasis**

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**Objectives:** To characterize the microbiota present in the lungs of patients with cystic fibrosis (CF) and non-CF related bronchiectasis (BE), and to determine changes in diversity and relative abundance during exacerbations and when patients were clinically stable.

**Methods:** Sputum samples from CF (n=48) and BE (n=28) patients were processed using culture dependent (strict anaerobic culture) and culture independent (454-FLX) methods. Agreement between the two methods, microbial diversity (Shannon-Wiener Index) and abundance within samples was determined.

**Results:** Correlation was apparent between the methods for quantitation of the main bacterial genera such as *Pseudomonas* spp. (r=0.46; P=0.015) and *Burkholderia* spp. (r=0.79; P=0.001) in CF, and *Haemophilus* spp. (r=0.71; P<0.001) and *Pseudomonas* spp. (r=0.80; P<0.001) in BE. Although rich microbial communities were observed within CF and BE airways, a small number of genera represented approximately 90% of all sequences. Diversity in patients predominantly infected with *Pseudomonas* spp. did not differ from diversity in the pooled samples for either of the cohorts (CF, P=0.28; BE, P=0.33). However, diversity was reduced in patient's predominantly infected by *Burkholderia* spp. (CF, P<0.001) and *Haemophilus* spp. (BE, P=0.0053).

**Conclusions:** There was a correlation between abundance of the predominant organisms by both methods, with both required to construct a comprehensive picture of the airway microbiota. Reduced microbial diversity was primarily related to the presence of *Burkholderia* spp. and *Haemophilus* spp. in CF and BE, respectively.

**105 The addition of broth culture increases the sensitivity of cough swabs for the detection of *P. aeruginosa* from people with CF**

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**Objective:** To determine whether the addition of acetamide nutrient broth (ANB) to our standard solid media-based method increased the isolation rate of *P. aeruginosa* from cough swabs.

**Method:** Between January 2011 and February 2011 323 cough swabs were examined using a standard solid media-based method. The tip of each cough swab was subsequently placed into ANB, incubated for 24 hours at 37°C, and sub-cultured onto solid media. The additional ANB sub-cultures were examined in a blinded fashion to the standard method cultures. All suspect isolates from either method were first provisionally identified using colonial appearance, oxidase reaction and API 20NE. All isolates suspected to be *P. aeruginosa* were confirmed by species-specific PCR.

**Results:** Overall, 39 (12%) cough swabs were positive for *P. aeruginosa* by either method. Only 23 were positive using the standard method whereas 35 were positive following pre-incubation in ANB. The sensitivity of cough swab culture for *P. aeruginosa* was higher following the use of ANB (90%) than by using solid media alone (59%).

**Conclusion:** The addition of ANB increased the sensitivity of cough swab cultures for the isolation of *P. aeruginosa* from people with CF.

**106 Non fermenters in CF samples. Ten years microbiology practice and results**

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During the period of 2002 to 2011, 267 patients with CF were followed in our outpatient clinic. In this period routine microbiological samples of the upper respiratory tract (URT) were processed based on ASM Manual and Vitek I. In 2006 BCSEA was introduced and LMG strains QC, and so, since 2010 Vitek II and PCR for routine inconclusive non-fermentative (NFB) bacteria. Among the 267 patients, 72% had at least one sample positive for *Pseudomonas aeruginosa* and 40% (108) had more than three positive samples in one year, 82% of these being characterized as chronic infections, 10% intermittent colonization and 8% of patients had eradication after chronic status. *Burkholderia cepacia* complex was isolated in 65 patients (prevalence 24%), with only 5 cases considered chronically infected (1.8%). For *Achromobacter* spp., 50% of all patients had at least one positive sample in URT, with only 6.6% (9 cases) with persistent colonization and only 3 patients maintain positive cultures for over 5 years. *Stenotrophomonas maltophilia* was isolated in 26% but only for 5 cases were persistent for >3 years, associated with severe pulmonary damage and other potential pathogens. Among other NFB that may be related to pathogenicity, from 14 patients were isolated *Chryseobacterium* spp., 6 *Cupriavidus pauculus*, 5 *Alcaligenes* spp., 5 *Elizabethkingia meningosepticum*, 4 *Bordetella bronchiseptica*, 4 *Ochrobactrum antropii*, 2 *Ralstonia* sp., 2 *Delftia acidovorans*, 1 *Burkholderia gladioli* and 1 *Rhizobium radiobacter*. Improved methods for identifying bacteria may clarify the pathogenic potential of these NFB, but it is still clear the predominance of *P. aeruginosa*, *B. cepacia* complex, *Achromobacter* spp. and *S. maltophilia* and its association with patients with poor lung function.

**107 Routine identification of bacteria from sputum samples of cystic fibrosis patients by MALDI-TOF**

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**Background:** Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry has emerged as a rapid and powerful tool for routine identification of bacteria in clinical microbiology. The objective of our study was to identify exhaustively all bacterial isolates growing in standard agar plates from sputum samples of Cystic Fibrosis (CF) patients.

**Methodology/principal findings:** From November to December 2011, we collected 80 sputum samples corresponding to 68 patients including 40 children and 28 adults in two CF medical center from Marseille, France. All samples were cultured on 4 specific agar plates including CNA (columbia colistin-nalidixic acid), PVX (chocolate PolyViteX agar), Mac Conkey agar, and Cepacia agar. All colonies retrieved from these plates were identified using MALDI-TOF mass spectrometry. Overall, 507 different colonies were collected (mean at 5.8 colonies per sample) leading to the identification of 58 different bacterial species. Many colonies not correctly identified were in fact yeasts that represent about 20% of the total colonies. Among the remaining isolates about 3 to 5% could not be identified even after re-isolation and correct identification by PCR amplification and sequencing is in progress.

**Conclusions:** Since MALDI-TOF is a fast and reliable method for bacterial identification, we believe that this technique associated with more extensive cultivation approaches will enhance the number of new and/or emerging bacterial species recovered from CF. This will help to understand the complexity and the dynamic of this complex microbiota during exacerbations as well as during antibiotic treatment.