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 presence of organisms by both methods, with both required to construct a comprehensive picture for characterizing community composition in the airways of patients with CF and BE.

Results: Correlation was apparent between the methods for quantification 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 for characterizing community composition in the airways of patients with CF and BE. Reduced microbial diversity was primarily related to the presence of Burkholderia spp. and Haemophilus spp. in CF and BE, respectively.

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, oxides 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.

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 BCSA 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 anthropi, 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.