179 Clinical Microbiology of *Haemophilus influenzae* in Cystic Fibrosis

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One hundred and twenty-four cystic fibrosis patients (lung transplant recipients excluded) delivered 998 respiratory tract samples during a 12 month period, and *Haemophilus influenzae* was isolated from 245 samples from 79 patients. *H. influenzae* was cultured at least once from 27 of 27 (100%) patients below 6 years of age, from 34 of 57 (60%) patients aged 6–17 years, and from 10 of 40 (25%) adult patients (⩾18 y). From 25 patients, *H. influenzae* was recovered from ⩾ half of their samples, and these patients accounted for 55% of all isolates. Cultivation of *H. influenzae* elicited treatment with oral amoxicillin (or amoxicillin-clavulanic acid; in rare instances ciprofloxacin), also in the absence of symptoms. Severe exacerbations were treated with intravenous cefuroxime or cetftriaxone. Fifteen percent of the isolates produced β-lactamase, another 12% exhibited decreased susceptibility to ampicillin (low-BLNAR (beta-lactamase negative ampicillin-resistant) *H. influenzae*), as evaluated by reduced susceptibility to oral cephalosporins by a disc-diffusion assay. These rates were similar to the susceptibility levels observed with *H. influenzae* cultured from non-CF patients. Whether the repeated isolation of *H. influenzae* from the same patient is attributable to a chronic colonization with a single strain is investigated by pulsed-field gel electrophoresis.

180* Detection of anaerobic bacteria in bronchoalveolar lavage fluid from paediatric CF patients

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Introduction and Aims: We have shown the presence of aerobic and anaerobic bacteria in adult CF patients when stable and during acute exacerbations. The aim of this study was to determine if anaerobic bacteria are present in the lower airways of paediatric CF patients and to determine their antimicrobial susceptibility.
Methods: Broncholaveolar lavage fluid (BALF) samples were collected from 33 CF children (mean age 9.4±0.9 yrs; range 1–19) at the University of North Carolina. For culture detection, samples were plated on selective agars and bacteria quantified by viable count and identified by PCR and sequencing of 16S rRNA genes. Antimicrobial susceptibility was determined using E-tests. For molecular detection, total DNA was extracted from BALF and bacterial species were identified by terminal restriction fragment length polymorphism (T-RFLP) analysis.
Results: No bacteria were detected in 5/33 (15%) and 7/32 (22%) samples by culture and T-RFLP, respectively. The most frequently isolated aerobic bacteria was *S. aureus* (meticillin-sensitive and resistant) which was detected by culture in 13/33 (39%) samples. Anaerobically growing bacteria were cultured in 13/33 (39%) samples with *Prevotella* (4 samples) and *Propionibacterium* (3 samples) species predominating. T-RFLP detected anaerobic bacteria in 13/32 (41%) samples. The anaerobes cultured were all susceptible to meropenem and piperacillin/tazobactam but some resistance was apparent to metronidazole, clindamycin and tobramycin.
Conclusion: Anaerobic infection may occur early in CF pathogenesis. Culture and molecular methods complement each other for detection of anaerobic organisms.
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181* Impact of strict anaerobs on the pathogenesis of lung infection in patients with Cystic Fibrosis

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CF patients produce large amounts of hypoxic/anaerobic plugs which may favour the growth of strict anaerobes. Here we show that 25–69% of investigated CF patients were infected with one or more anaerobs including Prevotella and Veionella subspecies. In 75% of the patients, Prevotella species were present in numbers of 10^5–10^7 CFU/ml sputum. Supernatant fluids of Prevotella intermedia produced cytotoxins for human respiratory epithelial cells and neutrophils. To assess whether P. intermedia contributes to the pathophysiology of CF lung disease, membrane proteins of P. intermedia strain 25611 were used in ELISA and Western blotting to detect specific serum antibodies. CF patients had increased serum antibody titers against P. intermedia compared to controls. Western blotting showed that antibodies bound to membrane proteins of P. intermedia, identified by MALDI-TOF as PIN AO373 and PIN AO107. These data suggest that cellular components of P. intermedia can contribute to the CF pathophysiology by immune complex formation thereby increasing inflammation. We also demonstrate that under anaerobic nutrient limitation, P. intermedia successfully competes with *P. aeruginosa* for growth in vitro and in a murine lung infection model. Taken together our data support previous investigations that CF sputum specimens contain anaerobic organisms. The data also suggest that strict anaerobic bacteria contribute to the pathophysiology of lung disease in CF patients.

182 Microbiological characteristics of *Achromobacter xylosoxidans* isolated from patients with cystic fibrosis

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Achromobacter xylosoxidans has been suggested as pathogen in lung disease of cystic fibrosis patients, but with no well established clinical relevance. We compared the manual versus automated microbiological identification and the susceptibility to antimicrobial agents for *A. xylosoxidans* strains isolated from pediatric CF patients seen at reference center at our State University Hospital. The microorganisms were identified by 33 conventional tests (ASM – 2007), Vitek II BioMerieux® and the LMG reference strains of *A. xylosoxidans*, *A. denitrificans* and *A. piechaudi* as control. The antimicrobial sensitivity was done by E-test®. For *Achromobacter* discrimination PCR assay for 16S rRNA was used. From the 33 tests applied, only reduction of nitrite to gas, OF glicose, OF xylose and growth at 42ºC were useful for species discrimination. Vitek II® could identify LMG 1231 *A. denitrificans* correctly, *A. piechaudii* LMG 1873 only at genera level and *A. xylosoxidans* LMG 1863 was misidentified as *B. bronchiseptica*. Comparing with manual identification, Vitek II® identified correctly only 5/14 (36%) strains as *A. xylosoxidans* and disagree with 9/14 (64%) identified as *A. denitrificans*. The Etest® MIC 50% and 90% and range results were respectively in μg/mL: Ceftazidime: 6/12 (2–>256), Ciprofloxacin: 2/>32 (0.75–>32), Meropenem:0.19/>32 (0.094–>32), Minocyclin: 2/3(1.5–6), Sulfatrim: 0.19/>32 (0.023–>32), Tygeciclin:1.5/3(0.38–6) and Polymyxin B:3/4(1.5–6). These results confirms the difficulty to identify this microorganism and the limited options of therapy drugs.
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