195 *Inquilinus limosus*: an easily missed emerging resistant respiratory pathogen in cystic fibrosis patients

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Inquilinus limosus is a new multiresistant pathogen isolated from the sputa of cystic fibrosis (CF) patients. Because this bacterium is not recorded in all commercial identification system databases currently available, Longitudinal study for *I. limosus* detection with a new real-time PCR assay with a Taqman* probe targeting the 16S rRNA gene was developed. From January 2006 to June 2007, 436 sputum samples recovered from 145 CF patients and 54 non-CF patients were screened blindly for the presence of *I. limosus*. Using our real-time PCR, we have detected 9 *I. limosus* positive PCR in sputa from four CF patients (2.8%) that were also culture positive in 8 cases. Interestingly, in one patient, *I. limosus* was detected using real-time PCR three months before the culture was positive. Specificity of our real-time PCR was verified using the programme BLAST and also using suspension of several pathogens recovered habitually in CF patients. For the sensitivity: the minimal numbers of CFU detectable was 2 CFU/PCR.
In conclusion *I. limosus* should be added in the list of emerging multiresistant pathogens retrieved in CF patients. Thus, we have developed a real-time PCR as a molecular method faster and easier than the amplification-sequencing for prompt detection of *I. limosus* with very good specificity and sensitivity.
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196 *Nocardia farcinica* isolation in CF sputum: therapeutic considerations

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Introduction: *Nocardia* spp. are seldom reported as pathogens for respiratory infection in CF. Significance and interpretation of such isolations have to be considered.
Case: We describe a 16 years old female with stable CF, colonized with *S. aureus* (MSSA) since more than 10 years. Nutritional status is normal with BMI = P50, and lung disease is moderate with stable lungfunction, FVC being 82% and FEV1 = 85% of predicted values. Recently, she presented with a moderate exacerbation of cough and increased sputum production, without fever and deterioration of lung function. Chest X-ray was stable. *Nocardia farcinica* was cultured together with *S. aureus*. Treatment with anti-staph antibiotics (amoxyclav) was effective. However, *Nocardia farcinica* was cultured again in 2 control sputum samples in a period of 3 weeks. Although there were no acute respiratory symptoms, the patient was commenced on cotrimoxazole (160 mg/800 mg, b.i.d.) for a period of 3 months.
Discussion: *Nocardia* spp. are aerobic Gram-positive bacteria, infecting mostly the respiratory tract of subjects with immunosuppression or with underlying pulmonary disease. Corticosteroids are important cofactors for infection. Nocardiosis is rarely described in CF, and this patient did not receive any steroids. Details for treatment indication in CF are not given. Motivation for treatment in CF is concern for possible dissemination and deterioration.

197 *Geosmithia argillacea* – a potential new pathogen in cystic fibrosis lung disease?

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Between January 2005 and June 2007 8 patients, from a total adult and paediatric population of approximately 500, were identified to regularly have a sputum culture positive for *Geosmithia argillacea* (GA). This fungus is known to be closely related to Penicillium species and was identified by ITS1 and LSU rRNA DNA sequence. Examination of archived fungal strains revealed that this organism was isolated from a CF patient (at a different institution) in 1991. GA has occasionally been isolated from respiratory specimens in other non-CF patients and from specimens suggesting possible involvement in deep-seated infections. Although GA is not recognised to be a pathogen in the CF lung its ability to grow at temperatures of up to 45ºC and its persistence in sputum cultures suggest it could be important particularly following lung transplantation.
Patients colonised with GA had a median age of 20 years (6–27), FEV1 of 56% (15–82) and duration of colonisation of up to 29 months. Over the same time period all affected patients had sputum specimens positive for *Aspergillus fumigatus*, 5 of 8 also had positive samples for *Pseudomonas aeruginosa* and *Mycobacterium* species, 2 of 8 had specimens positive for *Burkholderia cepacia* species and *Staphylococcus aureus*.
In the opinion of the clinicians caring for the patients who had GA in sputum specimens its appearance and persistence was not associated with a noticeable change in their clinical condition. However, a concern remains that should these patients become immunocompromised then GA may cause invasive disease.

198 Gut microbiota composition in cystic fibrosis patients: molecular approach and classical culture

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Objective: To analyze the gut microbiota composition of cystic fibrosis (CF) patients by classical culture and also molecular approaches.
Methods: Faecal samples from 18 CF patients and from 20 healthy volunteers (HV) were seeded in selective media for bacterial isolating. Total DNA was obtained from the faeces and quantitative-PCR experiments were conducted in order to know the bacterial density for the Bacteroides, Fusobacterium, and Lactic Acid Bacteria (LAB) groups. PCR-DGGE was performed with universal primers sets and also with the described bacterial groups.
Results: Lower bacterial charge was found in the CF faecal samples than in the HV. Curiously, Gram-negative bacteria were not detected in CF samples. Enterococcal and staphylococcal clones were detected only in 10 and 9 CF patients, respectively, whereas for HV these genera were represented in all subjects. Several LAB genera as Lactococcus, Lactobacillus, and Pediococcus were detected in almost 50% of the CF samples. Quantitative-PCR results showed differences among the density proportions of the bacterial groups in the CF patients versus the HV: Bacteroides (6:1), Fusobacterium (1.8:1), and LAB (2:1) groups. DGGE-experiments showed particular bands associated only to CF patients.
Conclusion: Significant differences were detected in the CF gut microbiota composition comparing with HV, being relevant the higher amounts of anaerobes and the absence of Gram-negative cultivable organism. These differences can be related with antimicrobial selective pressure and particular gut mucosal conditions in CF patients.