128 Isolation and identification of "difficult" pathogens from CF airways: molecular analysis results

P. Cocchi, N. Ravenni, G. Taccetti, S. Campana. *Cystic Fibrosis Center, Department of Paediatrics, University of Florence, Florence, Italy*

Introduction: An increasing number of Cystic Fibrosis (CF) pathogens results difficult to identify: unusual bacteria and novel species recovered from respiratory samples represent an additional challenge. Conventional tools for isolation and identification sometimes failed to give results, so that the prevalence and potential pathogenic role of these species remain unclear. Ribosomal DNA sequencing has greatly facilitated the identification of these bacteria, so that we investigate the utility of 16S rRNA gene (16S rDNA) sequencing.
Methods: We examined a total of 25 strains, grown on *Burkholderia cepacia* Selective Agar (BCSA) and Mac Conkey Agar (MCK) which were unable to be identified by traditional identification biochemical tests. DNA was extracted and, according to published protocols, we amplified a 1000 nucleotides portion of 16S rDNA. These nucleotide sequences were then determined and compared with GeneBank database.
Results and conclusions: 3 out of 25 strains are grown on BCSA (12%): 2 isolates belong to the Chryseobacterium genus and 1 to the *Burkholderia cepacia* complex (Bcc). 22 out of 25 strains are grown on MCK: 5 isolates identified as Bcc members (22.7%) failed to grow on Bcc specific medium (BCSA) so that they would be undetected; 3 as *S. marcescens* (13.7%); 2 as *P. aeruginosa* (9%); 4 as *S. malthophilia* (18.3%), 4 as *A. xylosoxidans* (18.3%), 1 as *P. agglomerans* (4.5%), 1 as *Acinetobacter sp* (4.5%), 1 as *Chryseobacterium sp* (4.5%) and 1 as *Enterobacter sp* (4.5%). This kind of approach allows to identify both emerging pathogens and unusual isolates and improves our undestanding about the complexity of bacterial community within CF lung.

129 Molecular epidemiology of the longitudinal course of the *Pseudomonas aeruginosa* infection in Cystic Fibrosis

N. Cramer[1], L. Wiehlmann[1], G. Wagner[2], B. Tümmler[1]. [1]*Klinische Forschergruppe OE 6711, Medizinische Hochschule Hannover;* [2]*Clondiag Chip Technologies, Jena, Germany*

More than 350 sequential *Pseudomonas aeruginosa* isolates which were retrieved from 36 cystic fibrosis (CF) patients since the onset of colonization over an up to 20-year period were genotyped by a high-throughput DNA chip that allows the identification of the *P. aeruginosa* genotype by SNP-typing and the repertoire of the accessory genome. In order to gain insight into the adaptation of *P. aeruginosa* to the CF lung habitat, the following issues were addressed: are there changes in the core genome or the gene islands; how long does a clone persist in the lung; are there stable co-colonisations and is there any correlation between the clone and the clinical status of the colonised patient?
Results. Fourty-two percent of the analysed patients had only one clone during the whole observation time, whereas the other 58% were co-colonized with more than one clone at at least one point of time. The SNP-pattern was more conserved than that of the gene islands implying that the evolutionary clock of the core genome runs at a slower speed than that of the islands. By year 6 only 50% of the initial clones were still detected in the patients' airways. Most co-colonisations were observed between the first four years. Radiations (microevolution) occurred more often in alive patients than in patients, who deceased during the study. Therefore, we propose that bacterial genome remodelling leads to a better adaptation between the host and the opportunistic pathogen. Furthermore, we suggest that radiation decreases the host immune response and leads to a milder course of lung disease in CF.

130 Characteristics of a new transmissible multiresistant *Pseudomonas aeruginosa* strain with a unique genotype

P. Meyer[1], A.-C. Petersson[2]. [1]*CF-unit, Pediatric clinic,* [2]*Clinical Microbiology and Immunology, University Hospital, Lund, Sweden*

Background: Epidemiological- and pulsed field gel electrophoresis (PFGE) data strongly indicates transmission of a multiresistant *Pseudomonas aeruginosa* (Pa) strain at a winter camp for CF-children in Sweden 2005. PFGE analysis showed a unique pattern assigned J-strain. This pattern was identical for strains from other patients attending the same camp but living in different parts of Sweden (unpublished data). Segregation and hygiene routines were clear cut and respected. The transmission route is unknown.
Aim: The aim of the study was to characterize this new transmissible Pa J strain by sequence analysis and susceptibility testing and compare it to known transmissible Pa genotypes.
Methods: Species identity was verified by sequence analysis of 16S rDNA. Molecular characterization was performed by multilocus sequence typing (MLST) according to Curran et al (J Clin Microbiol 2004, 42: 5644–49). Antibiotic susceptibility testing was performed using the disc diffusion method (Oxoid) and E-tests (AB Biodisk).
Results: MLST was performed in two steps, where the first step included analysis of three loci in order to screen for known epidemic strains. The analysis show that the Pa J strain differs from all known epidemic strains described so far.
The antibiogram showed susceptibility for colistin but resistance to all other tested antibiotics (meropenem, aztreonam, piperacillin/tazobactam, gentamicin, tobramycin, ceftazidime, ciprofloxacin).
Conclusion: A new multiresistant transmissible strain of Pa has been identified. MLST indicates a unique genotype not related to previously described epidemic strains.

131 Survey of *Pseudomonas aeruginosa* (PA) genotypes in a regional paediatric shared care CF service

H. Wyatt[1], M. Graver[2], G. Ruiz[1], J. Wade[2], T. Pitt[3], J. Price[1]. [1]*CF Unit,* [2]*Dept. of Microbiology, King's College Hospital,* [3]*Centre for Infections, HPA, London, UK*

Aim: To determine the prevalence of clusters of PA with the same genotype and thereby whether the shared care system promotes effective infection control.
Methods: Any patient attending one of the nine clinics in the King's College Hospital Regional Paediatric CF service was included. These outreach clinics were held at the patients' local hospital and contained between 7 and 20 children. Segregation by culture results was practised in all clinics. PA samples from cultures collected over a 17 month period were genotyped by Pulsed Field Gel Electrophoresis at the regional reference laboratory.
Results: 150 patients (75M), median age(range) 9.35 y(0.45–18.25). 21 (14%) patients had never cultured PA. 94 (63%) of the others had at least one PA positive culture during the collection period and PA from 69 of these patients was sent for genotyping (typing unsuccessful in 4); the rest of the patients either had only one PA positive culture, which was not saved inadvertently (23), or no positive culture (30) in that time.
Most (77%) PA genotypes were unique. Seven different PA genotypes were seen in 15 unrelated patients. Three patients had Liverpool Epidemic Strain (LES); two of these attended the same clinic and also mixed socially. Six other pairs of children shared a PA with same the genotype, five of the pairs were geographically quite separate. The sixth pair attended the same shared care clinic.
Conclusion: There was little evidence of cross infection of PA across the region. The cohorting of patients by this system of shared care clinics may reduce the risk of transmission of respiratory tract organisms. Segregation of patients within their own clinic remains essential.