128 Population structure of *Pseudomonas aeruginosa* from cystic fibrosis patients

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Background: PFGE and MLST are molecular typing tools to know the genetic relation among bacterial isolates. Classically, PFGE has been the gold-standard, whereas data about MLST in CF isolates still remained unknown.

Material and Methods: 42 *P. aeruginosa* isolates from 10 CF-patients were included. PFGE-*Spe*I was applied with UPGMA dendrogram construction. One isolate per each pulsotype was further typed by MLST following the recommendations website http://pubmlst.org/paeruginosa/.

Results: 40 of the 42 CF *P. aeruginosa* isolates were clustered in 18 PFGE patterns and 2 isolates (21 and 36) were consistently untypeable. One patient presented four pulsotypes among 9 isolates and other has three pulsotypes, whereas the most common situation was the existence of two pulsotypes (6 patients). Finally, only two patients have a unique PFGE-pattern. Twenty-two isolates were selected for MLST typing, including the two previously untypable by PFGE and at least one isolate per patient and pulsotype. High genetic diversity was found, being ST242 the most prevalent lineage (4 isolates, two patients). ST17 and ST809 grouped three isolates and two patients each one, and ST620 was found in two isolates from two patients. The other ST detected in CF-isolates only grouped one isolate each one and were: ST996, ST360, ST274, ST980 and two new STs.

Conclusion: A high genetic diversity by MLST was found in our CF-*P. aeruginosa* isolates, demonstrating independent acquisition. Although PFGE is considered the gold-standard, the MLST tool is useful to determine the population structure, demonstrating genetic relationship between bacterial isolates.

129* Comparison of clinical and environmental *Pseudomonas aeruginosa*: evidence for recombination

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Objectives: *P. aeruginosa* (*Pa*) is an environmental organism capable of causing opportunistic infections in humans and animals, and is the most common bacterial pathogen in cystic fibrosis (CF). Earlier studies have demonstrated a non-clonal epidemic population structure and some evidence of recombination. However, the phylogenetic analysis approach taken previously may be inaccurate when applied to freely recombining bacteria. Using multilocus sequence typing (MLST) data, we estimated the population structure and rate of recombination among 501 environmental, CF, non-CF & animal *Pa* isolates.

Methods: Evolutionary patterns of descent were determined by eBURST and also compared with the *Pa* MLST database (995 sequence types [STs]). The population recombination rate was estimated using a composite likelihood method (Ldhat 2.1), and molecular events giving rise to single- and double-locus variants (SLVs & DLVs) within clonal complexes (CCs) were used to estimate the contribution of recombination to recent clonal divergence.

Results and Conclusion: We established 6 CCs consisting of ⩾3 STs, 26 BURST groups of 2 STs, and 197 singletons. Pairwise sequence analysis revealed recombination occurred 19.5 times more frequently than mutation. Examination of allelic changes amongst SLVs & DLVs associated with the initial stages of clonal diversification also revealed higher rates of recombination compared to mutation. These data indicate evolutionary change is more likely to occur by recombination than mutation, thus obscuring the phylogenetic signal. Techniques such as eBURST, which associate on the basis of allelic designation, are appropriate for determining evolutionary relationships for *Pa*.

130 Prevalence of TSST-1-, PVL- and SEA-producing *Staphylococcus aureus* in cystic fibrosis patients

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Staphylococcus aureus is a well-known pathogen in CF but its virulence gene content remains unevaluated in this context outside the recent report of emerging Panton-Valentine Leukocidin (PVL)-positive methicillin-resistant *S. aureus* (MRSA) in a few centers. The aim of this study was to characterize the toxin-encoding gene content, focusing on PVL gene (*pvl*), toxic shock syndrome toxin 1 gene (*tst*) and enterotoxin A gene (*sea*), in 130 non-redundant isolates from 104 CF patients. Isolates were also screened for antibiotic resistance, *mecA* gene, *SCCmec* and *agr* types. The *pvl*, *tst* and *sea* genes were detected in 0.8% (n = 1), 14.6% (n = 19) and 21.5% (n = 28) of the isolates, respectively. MRSA accounted for 22% of the strains (n = 29), *SCCmec* of type IV and IVE being mainly observed. Although not fully characterized here, most MRSA strains showed the characteristics of the pandemic hospital-acquired Pediatric clone (*agr-2*, TSST-1, *SCCmec* type IV). *pvl* gene was detected in a MSSA isolate. The high rate of superantigenic toxin-producing *S. aureus* demonstrated in this descriptive study detecting only 3 toxin-encoding genes, questioned about their implication in the CF respiratory status and the most appropriate treatment to be instaured. We are currently reviewing the clinical status at strain's isolation in our population but case control studies evaluating both the impact of such strains on pulmonary exacerbation and the potential benefice of adding antibiotics with antitoxinic activity remain needed.

131 Selective isolation, morphotyping and S*pa*-typing of *Staphylococcus aureus* in respiratory tract samples from patients with cystic fibrosis

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Objectives: Our routine detection of *Staphylococcus aureus* in cystic fibrosis (CF) specimens rely on recognition of characteristic colonial morphology on various agars, primarily 5% horse blood agar.

Methods: We tested the use of selective media by parallel processing of clinical samples in the routine laboratory and by blinded culturing on *S. aureus* ID agar (bioMériuex) and 6, 5% NaCl broth. The samples were also cultured on Columbia blood agar and Schaedler agar, and occurrence of morphological variants was assessed by careful observation of differences in haemolysis, pigmentation, size and consistency of single colonies. Individual colonies of *S. aureus* in the same specimen were considered variants if they differed in at least one of these criteria. During a period of three months 311 samples from 134 CF patients were prospectively investigated. *S. aureus* was detected in 135 samples (43%) by the routine method, and in 172 samples (55%) by use of selective media, corresponding to a 27% increase in the overall *S. aureus* detection rate. Between one and six different morphotypes of *S. aureus* were observed per sample, and a total of 297 morphotypes were characterized from 172 samples. In 54% of the samples only one morphotype of *S. aureus* was observed, while 10 samples (6%) contained more than 3 morphotypes. Haemolysis was rarely observed on 5% horse blood agar, while 254 morphotypes (86%) expressed different degrees of beta-haemolysis on Columbia sheep blood agar.

Conclusion: Our data show that the additional use of selective media significantly increased the detection of *S. aureus*. The genetic significance of morphological variability is being investigated by *spa*-typing.