Investigation of a shared strain of *Burkholderia cenocepacia* isolated from two CF siblings

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Two siblings with CF attending the CF clinic in SVUH are both infected with *Burkholderia cenocepacia* IIIA. In one host the organism remains confined to the lungs while in the other host, the organism has been isolated both from lungs and blood cultures during exacerbation.

**Objectives:**

1. To investigate whether there are any genetic differences between the sputum isolates from both hosts and between the blood and sputum isolates in the one host.
2. To examine differences in the pathogenicity of these blood and sputum isolates over time in lung epithelial cells, *in vitro*.

**Methods:** Serial *Burkholderia cenocepacia* isolates were collected from the two siblings.

- Restriction Fragment Length Polymorphism (RFLP), pulse field gel electrophoresis (PFGE) and recA sequencing was carried out on all isolates.
- Intracellular invasion of the isolates into CF bronchial epithelial cells (CFBEs) was examined by antibody protection assay. Translocation across polarised cell monolayers was also examined.

**Conclusions:**

- We confirmed the genomovar type of the organism by both RFLP and recA sequencing. All isolates were identical by PFGE typing. RecA sequences did not show any differences. Additional molecular investigations on isolates from both hosts will include analysis of seven house keeping genes by MLST to look for any genetic divergences.
- Comparison of respiratory and blood isolates invasion into CFBE cells isolates is ongoing. We will also compare the proteome of these isolates by MALDI-TOF; to examine if differential protein expression is involved in the different infection profiles in the two siblings.

Statins, a widely used cholesterol-lowering drug, modulate key virulence behaviour of *Pseudomonas aeruginosa*

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Statins are a class of medicine which reduces cholesterol biosynthesis by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase. Although known pleiotropic effects of statins include anti-inflammatory and anti-microbial activity, the effect of statins on bacterial behaviour has not yet been fully investigated. We therefore investigated the effect of simvastatin (SIM), lovastatin (LOV), and mevastatin (MEV) on a range of key virulence factors of *Pseudomonas aeruginosa*, the main pathogen associated with mortality and morbidity in cystic fibrosis (CF) patients. Firstly, anti-microbial activity of statins on the *P. aeruginosa* model strains PA01 and PA14 was assessed. None of the statins affected the growth of *P. aeruginosa*, and they did not have an impact on quorum sensing and type three secretion. However, statins reduced motility and biofilm formation, which are crucial factors involved in the colonisation and persistence of infections in the lungs of CF patients. Altogether, these data demonstrate that statins have a specific, targeted effect against *P. aeruginosa*. Further studies will focus on elucidating the molecular mechanism by which statins alter *Pseudomonas* pathogenicity. The dual influence of statins on inflammation and pathogen behaviour highlights the potential for their use in the development of innovative therapeutic strategies.

Do swarming and twitching motility in *Pseudomonas aeruginosa* have a role in early infection of cystic fibrosis airways and in the enhanced transmissibility of epidemic strains?

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**Objectives:** We investigated the hypothesis that swarming and twitching motility in *Pseudomonas aeruginosa* have a role in early CF lung infection and in the enhanced transmissibility of epidemic strains.

**Methods:** Three panels of *P. aeruginosa* isolates were investigated. (A) 46 isolates from natural environments; (B) 21 first sputum isolates obtained during longitudinal studies. (C) 17 isolates representing epidemic strains. Bacterial twitching and swarming were investigated using soft agar and other appropriate conditions. Bacterial coloniality was excluded by pulsed-field gel electrophoresis.

**Results:** *Pseudomonas aeruginosa* PA01, used as a control, showed reproducible twitching measuring 23 mm. The majority of environmental isolates (37/46, 80%) also exhibited twitching (range 5–23 mm, mean 15.1 mm). Amongst first isolates, twitching was observed in 11/21 isolates (52%; range 5–27 mm, mean 16 mm). In the 17 epidemic strains, twitching was restricted to 4/17 isolates (24%; range 4–16 mm, mean 8.8 mm). Swarming motility was measured qualitatively by the presence or absence of tendril-like growth originating from the point of stab inoculation. The percentage of isolates which exhibited swarming in panels A, B and C were 24/46 (52%), 4/21 (19%) and 0/17 (0%) respectively. Twitching and swarming were independent of mucoidy or antibiotic resistance.

**Conclusion:** Twitching and swarming motility appear to have a negative influence on initial *Pseudomonas* infection of CF airways and as an explanation for enhanced transmissibility. Since these forms of motility are considered to play an important role in other respiratory infections our findings are unexpected and merit further study.

Role of *Nocardia* in patients with cystic fibrosis: acute infection, chronic infection or colonization?

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**Objective:** Clinical impact of *Nocardia* isolated in patients with cystic fibrosis (CF patients).

**Method:** *Nocardia* spp. were isolated from the lungs of 10 CF patients (6 male and 4 female, aged 7 to 46) in the CF Centers of Lyon and Toulouse, France, between 1997 and 2011. Clinical data (risk factors, clinical status at first recovery, symptoms, Shwachman and Brasfield scores, FVC, FEV1, antimicrobial therapy) as well as bacteriological data (number of *Nocardia*-positive samples, antibiotic susceptibility, associated pathogens) were analyzed.

**Results:** Five species of *Nocardia* were identified: *N. translucens* (1), *N. asteroides* (1), *N. abscessus* (2), *N. farcinica* (2) and *N. cyriacigeorgica* (4). Two of the ten patients presented with exacerbations at first recovery. Eight patients were more or less symptomatic with few rales to severe dyspnea, purulent sputum or nodules. In seven out of the 10 samples, *Nocardia* was associated with other pathogens: *Staphylococcus aureus* (6 samples), *Pseudomonas aeruginosa* (3 samples), *Stenotrophomonas maltophilia* (2 samples), *Streptococcus pneumoniae* and *Haemophilus influenzae* (1 sample). Yeasts and molds were also observed. In 8 of the 10 patients, several samples (2 to 5) were *Nocardia* positive. Cotrimoxazole, β-lactams, aminoglycosides, linezolid, quinolones were used for treatment, systematically or in case of exacerbation, as single agents or in combination and for durations depending on the severity of symptoms.

**Conclusion:** The role of *Nocardia* in CF patients is poorly studied. Our 10 cases confirm that these bacteria can be responsible for asymptomatic and short colonizations, as well as for infections.