Comparison of biofilm formation between *Burkholderia cepacia* complex (Bcc) and *Pseudomonas aeruginosa* in the presence or absence of mucin

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We investigated the ability of three pathogens (*Burkholderia* multivorans, *Burkholderia cenocepacia*, and *Pseudomonas aeruginosa*) involved in chronic bacterial infections in the CF lung to form biofilm in the presence of mucin. Previously Landry et al. (2006) have shown that *P. aeruginosa* developed larger aggregates when grown on mucin compared to when grown on glass. We investigated the differences in biofilm-biomass (BFBM) for a total of fifty isolates belonging to all three species when grown in vitro on the presence of porcine stomach mucin.

Type III. Our results showed that on average biofilm formation by *B. cenocepacia* (n=17) (p<0.0001) and *B. multivorans* (n=11) or *B. cenocepacia* (n=17) (p<0.0001). Further investigations within members of *B. cenocepacia* demonstrated that members of the recA type-IIIA (n=6) formed on average significantly higher BFBM than other *B. cenocepacia* isolates (n=11) (p<0.0001). Further comparison of the average BFBM formation for isolates belonging to the highly problematic ET-12 clonal complex (n=4) and isolates belonging to *P. aeruginosa* (n=22) demonstrated that members of the ET-12 clonal complex formed on average comparable BFBM as isolates belonging to *P. aeruginosa* (p<0.79).

This study demonstrates that members of *P. aeruginosa* form on average significantly higher BFBM compared to members belonging either to *B. multivorans* or *B. cenocepacia*. However, members of the ET-12 clonal complex were observed to form on average higher BFBM than other members of *B. cenocepacia* and comparable BFBM production of *P. aeruginosa* when grown in vitro.

Clonally identical *Burkholderia cepacia* complex (Bcc) sequence type strain ST32 isolated from cystic fibrosis (CF) patients in French western Brittany compared to epidemiology of Bcc strains in France using Multilocus sequence typing (MLST)

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**Objectives:** To study the Bcc sequence type (ST) strains involved in CF patients in Roscoff compared to other centres in France (Brest, Saint-Brieuc, Rennes, Nantes, Bordeaux, Nîmes, Strasbourg, Clermont-Ferrand, Lyon, Marseille, Nice, Paris). Methods: Bcc isolates were obtained from 80 CF patients between 1994 and 2008 and identified using ReCA PCR-RFLP in Toulouse observatory centre. ST analysis was assessed by MLST in Brest. Results: Among the 80 Bcc isolates, 59 were identified B. cenocepacia, 20 B. multivorans and 1 B. stabilis. Among the 59 B. cenocepacia, 22 were identified as ST32 strains after MLST analysis; 15 of them were isolated in Roscoff out of 18 B. cenocepacia. ST32 strains were also isolated in Nancy (3/3) and sporadically in Bordeaux, Rennes, Nîmes, Lille and Paris. Other ST strains were identified as such as ST 31, 122, 159, 234, 241, 279 (with 1 or 2 strains in each ST) in the different French centres, without specific localisation. Conclusion: We found in this study a clonally identical Bcc ST32 strain (83%) isolated from CF patients followed in Roscoff (western Brittany). Since ST32 strain is the only one among ST strains of the IHA genomovar group to be found in matched clinical-environmental strains (Baldwin et al 2007), identification of this ST32 strain around Roscoff centre is ongoing. Supported by: Support and grant from Vaincre LA Mucoviscidose (2007–2008).

Congo Red staining of CF-related pathogens grown as biofilms in the absence or presence of mucin

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We investigated the ability of the three pathogens (*Burkholderia* multivorans (Bm), *Burkholderia cepacia* (Bc), and *Pseudomonas aeruginosa* (Pa)) involved in bacterial infections of the CF lung to form biofilm in vitro in the presence or absence of mucin. Previously, Harrison-Balestra et al. (2003) showed that Congo Red (CR) staining was a useful tool to visualise biofilm formation of isolates recovered from human burn wounds. Here CR staining was used to visually compare biofilm formation in the absence and presence of mucin.

Initial screening of a selection of isolates (n=6), from all three species, showed a significant increase in biofilm-biomass on microtitre plates, pre-coated with porcine stomach mucin Type III (p<0.0001), as shown by Crystal Violet staining. Three clinically relevant isolates; Bm (C1576, Glasgow epidemic strain), Bc (J2315, ET-12), and Pa (PVO219, Liverpool epidemic strain) were further analysed by CR staining. In the presence of mucin, all three isolates formed larger and more compact cellular aggregates than when grown in the absence of mucin. Without mucin, bacterial isolates demonstrated thin, disparate formations comprised of only a few bacteria. This study demonstrates that mucin influences the ability of bacteria to adhere to the surface and form biofilm aggregates. The application of CR staining allows visualisation of biofilm formation. As mucin is a relevant molecule within the CF lung environment, it's inclusion as a substrate for biofilm production may generate more useful results than using plastic alone. The results indicate that mucin-coated plates may be a more optimal model when investigating biofilm formation of CF-associated bacterial isolates.

*B. cepacia* cenocepacia: nearing the end?

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Many CF patients in the UK became chronically infected with the highly transmissible and often fatal pulmonary pathogen, *Burkholderia cepacia* (Bc), in the early part of the 1990s, following its transfer into the UK CF community from visitors to North American Summer Camps in the late 1980s. However, effective segregation and better microbiological surveillance has prevented further spread of this epidemic. When our adult unit opened in 1993, we accepted a cohort of these patients mainly from the paediatric sector and now report the outcome for our Bc patients, 16 years later. All 41 (mean age 28 years [range17 to 44], mean length of infection 87 months [1 to 180], 21 male) were strictly segregated from those without Bc infection. So far, 32 (78%) of these have died: 22 (69%; mean age 25 years [17 to 34], mean length of infection 64 months [1 to 144], 11 male) due to a fulminant necrotising pneumonia (the cepacia syndrome), and the remaining 10 (31%) due to respiratory failure caused by progressive lung damage (mean age 29 years [21 to 44], mean length of infection 68 months [10 to 135], 5 male). Of those still alive (mean age 36 years [24 to 43], mean length of infection 163 months [72 to 180], 5 male), there has been little alteration in clinical state over the last 3 years (mean FEV1 change -1.5% per year), suggesting they are a ‘survivor’ population. This group includes one CF person who visited the original summer camps in 1988, and whose clinical state remains unchanged, 21 years later. These data indicate that the epidemic of Bc in Merseyside is coming to an end, through the inevitable increased mortality caused by this organism coupled with the prevention of new cases by effective strict segregation policies in both adult and paediatric sectors.