

**105 Phenotypic and genotypic characterization of a cystic fibrosis outbreak strain of *Burkholderia multivorans***

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*Burkholderia multivorans*, a member of the *Burkholderia cepacia* complex (Bcc), is an important opportunistic pathogen of the cystic fibrosis (CF) lung. Although generally considered less virulent than the closely-related *B. cenocepacia*, certain strains of *B. multivorans* have been associated with significant morbidity and mortality. One such strain is the 'Glasgow outbreak strain' (represented by *B. multivorans* C1576), which was responsible for an outbreak within a paediatric CF unit in Glasgow (UK) in the early 1990s. By applying a variety of phenotypic and genotypic methods, including whole genome sequencing, we have investigated the genomic content and virulence of the Glasgow strain, and investigated its current prevalence in the UK. We report that the Glasgow strain remains within the UK's CF population, albeit at a low level. Several phenotypic changes are apparent within sequential isolates from individual patients, most notably a transition from mucoid to non-mucoid, and a loss of motility. Whilst the strain has undergone considerable genomic rearrangement since the original outbreak, the actual genomic content appears largely conserved. Several putative virulence factors have been identified within the genome, including filamentous haemagglutinin and a type IV secretion system (T4SS) that is distinct from other Bcc T4SSs. Ongoing studies aim to define the role of these putative virulence factors during infection.

**106 Siderophore expression by *Burkholderia cenocepacia* in response to changes in growth environment**

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Iron acquisition plays an important role in the survival of bacterial pathogens in the host environment. *Burkholderia cenocepacia*, an opportunistic pathogen in CF, produces at least five siderophores to sequester iron: ornibactin, pyochelin, cepabactin, cepaciachelin and salicylic acid, with ornibactin being the most important. We have examined the production of siderophores and the expression of siderophore related genes by *B. cenocepacia* J2315 in response to altered iron concentrations and established the time course of expression in response to iron depletion. Siderophore levels were determined by a quantitative CAS microassay. *B. cenocepacia* J2315 produced up to 80uM siderophore when iron concentrations were below 3uM in MM9 media compared to <20uM at higher iron concentrations. An examination of the genes involved in ornibactin synthesis (*orbA*, *orbF*) and its receptor (*pvdA*) in addition to the *pchD* gene for pyochelin synthesis and the pyochelin receptor (*fptA*), showed significant upregulation of these genes below 3uM iron. The activation of the three ornibactin related genes was more than 10 fold that of the pyochelin related genes, confirming the importance of this siderophore for *B. cenocepacia*. Furthermore, *B. cenocepacia* J2315, when transferred from an iron replete environment (3uM iron) to an iron depleted media, responded rapidly by expressing all three ornibactin related genes within 15 mins and reached maximal levels within 45 mins. These data demonstrate the sensitive and rapid adaptability of these organisms to iron availability. The potential impact of Bcc siderophores on lung epithelial cells is currently being examined.

**107 Chromogenic versus cetrимide-based media for *Pseudomonas aeruginosa* isolation**

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*Pseudomonas aeruginosa* (Pa) is the most common and important pathogen colonizing the lungs of cystic fibrosis (CF) patients. Detection of Pa colonization is usually achieved by culturing respiratory samples onto media such as MacConkey and cetrимide-based (CET) agar. Since the production of typical Pa pigment is frequently absent on commonly used media, Pa colonies may be indistinguishable from other non-pigmented Gram-negative strains. We compared the performance of Pa isolation using two different media, a classic selective agar and a chromogenic medium. Seventy-one respiratory samples from 71 CF patients were cultured on CET medium and on a Pa chromogenic (PA-ID) agar. All morphological variants from each medium were identified using biochemical and molecular methods. Thirty-eight out of 71 (53.5%) patients were Pa positive. CET agar recovered Pa from 37 out of 38 (97%) samples, while PA-ID agar recovered Pa from 30 out of 38 (79%) samples. Mucoid Pa was isolated from 22 out of 37 (59%) and from 8 out of 30 (27%) on CET agar and PA-ID agar respectively. We isolated Pa on PA-ID agar but not on CET agar only from one sample. 32% of samples showed from 1 to 4 different morphological colonies on CET agar, while only 16% of samples showed from 1 to 3 colony variants on PA-ID agar.

The Pa colored colonies on PA-ID agar are easier to distinguish, but alginate production and the number of morphological variants seem to be reduced. In contrast, we recovered a higher number of colony variants and increased exopolysaccharide production on CET agar. In conclusion, PA-ID agar may be used alongside CET agar, to identify colonies with atypical morphologies more quickly.

**108 Rapid diagnostic tests for the presence and fluctuations in the levels of *Pseudomonas aeruginosa* in cystic fibrosis patient sputum and cough swab samples**

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Once *Pseudomonas aeruginosa* is established in the cystic fibrosis (CF) lung, it is impossible to eradicate. Current diagnostic techniques rely on conventional culture, which is time consuming and subject to individual interpretation. In addition, the sensitivity has been questioned. Rapid diagnostic tests are key in the fight against chronic *P. aeruginosa* infection. Early identification of pathogens before an infection is established can lead to aggressive treatment to eradicate or delay chronic infection.

We have devised real-time quantitative PCR tests to detect (1) all bacteria, (2) *P. aeruginosa* and (3) the Liverpool Epidemic Strain (LES). We compared three approaches to *P. aeruginosa* quantitation in 22 CF patient sputum samples (A. real-time PCR of QIASYMPHONY-extracted DNA; B. Real-time PCR of column-extracted DNA and C. Culture). Real-time PCR assays were consistently more sensitive than culture techniques. There was no significant difference between the column-based and QIASYMPHONY extraction methods. A larger study based on 100 CF sputum and cough swab samples that have been processed using routine methods will be used to validate the assays.

Rapid high throughput DNA extraction directly from sputum samples reduces the time taken from sample to result from days to 4–5 h. Since actual and relative (to total bacteria) levels of *P. aeruginosa* can be detected, these tests could be used to rapidly determine whether antibiotic treatment is successful in reducing and/or eradicating *P. aeruginosa*.

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