52 *Burkholderia cenocepacia* shows increased attachment to cystic fibrosis lung epithelial cells over time of colonisation

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Objective: *Burkholderia cenocepacia (Bc)* is a highly antimicrobially resistant pathogen, which is rarely eradicated from cystic fibrosis (CF) patients. While *Bc* is generally confined to the lung, bacteraemia can develop in a subgroup of patients contributing to a sharp decline in health. How *Bc* chronically colonises and causes bacteraemia remain poorly understood. Bacteria can change phenotype during infection, adapting to the host environment. We aim to identify the alterations in *Bc* phenotype from initial colonisation to chronic infection.
Methods: A series of genetically identical sequential *Bc* isolates from two siblings with CF, one with chronic infection confined to the lung (Pt1), the other with bacteraemia (Pt2), were studied. *In vivo* virulence using *Galleria mellonella*, mucoidy on yeast extract mannitol (YEM) agar and adhesion to CF lung epithelial cells (CFBE41o–) were examined.
Results: All isolates were relatively avirulent *in vivo* in *Galleria mellonella*. The blood isolates from Pt2 were considerably more virulent than a sputum isolate from that patient ($P = 0.014$). Mucoid production by *Bc* has been associated with better patient outcomes; however, all but one isolate were non-mucoid on YEM. Both sputum and blood isolates increased attachment to CFBEs over time of colonisation ($p < 0.005$ and 0.001 respectively), indicating an increased potential for host epithelial interactions over time. This key adaptation was confirmed by confocal microscopy. We are currently comparing the proteomes of all isolates to identify alterations in protein expression that may be involved.
Conclusions: It is clear that *Bc* adapts and increases virulence during colonisation.

53 Genome changes leading to persistence of *Pseudomonas aeruginosa* in the airways of CF patients

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Objectives: Identification of genetic markers for transformation of intermittently colonizing *P. aeruginosa* (PA) to chronically infecting bacteria in CF lungs.
Methods: Clinical isolates of PA have been systematically collected for 10 years from early infection stages in CF children and young adults. For this study, 34 patients and therefrom ca. 500 isolates were chosen for full genome sequencing. Based on bioinformatics analyses, mutations accumulated during airway colonization were listed and a number of repeated patho-adaptive mutations identified.
Results: Approximately 75% of all patients were colonised or infected with the same clone type during the entire period of infection. Clones could be divided into two groups: persisting and non-persisting with a median period of residence of 4.25 years and 1.7 years, respectively ($p = 0.02$). Among all sequenced genomes we found 56 genes to be mutated more that 6 times suggesting a role in patho-adaptation; however only 18 genes remained consistently mutated in the persisting clones. These mutations mainly belong to: Envelope, Antibiotic Resistance, and Iron acquisition. We compared the time of appearance of mutations to colonisation time in persistent and non-persistent clones and found no significant difference between the two groups ($p = 0.7$).
Conclusion: Despite intensive antibiotic chemotherapy whenever PA is cultured from the CF lung, the bacteria are only eradicated in few patients. We think that adaptation probably happens before PA has established high enough densities in the lungs to be cultured from sputum or endolaryngeal suctions.

54 Intraclonal genome diversity of the major *Pseudomonas aeruginosa* clones C and PA14

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Pseudomonas aeruginosa clones C and PA14 are the two most prevalent clones in the bacterial population in both environment and disease habitats including cystic fibrosis (CF) lungs. Since no whole genome sequence of any clone C isolate is yet available, we sequenced the CF isolate NN2 as the future reference genome of *P. aeruginosa* clone C. The 6.9 Mb large genome was characterized in its genetic repertoire of protein-coding ORFs and of non-coding RNAs. To analyze the worldwide genomic diversity within these two clones, we sequenced the genomes of 58 clone C and 42 clone PA14 isolates from environment, acute infections and chronic airway infections in individuals with COPD or CF. The individual strains differ in the repertoire of the accessory genome and a median sequence diversity of 0.02% or 0.003% from each other. Protein variants with non-conservative amino acid substitutions were predominantly seen in elements of horizontal gene transfer and transporters and enzymes encoded by cargo region of the accessory genome indicating diversifying selection. Functional protein variants were very rare in the core genome in all strains but isolates from CF lungs. The CF strains accumulated pathoadaptive mutations in major regulators of the biofilm lifestyle and pilus and exopolysaccharide biogenesis genes indicating positive selection in loci that facilitate persistence in CF airways. Clone C or clone PA14 isolates of matching pyocin and antitoxin capabilities were tested for their competitive fitness in planctonic cultures for five days. The CF isolates were not significantly impaired in persistence compared to isolates from acute infections or inanimate aquatic habitats.

55 Investigation of the RetS–GacAS regulatory network in clinical *Pseudomonas aeruginosa* isolates reveals evolutionary adaptation

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Objectives: It is well documented that *P. aeruginosa* adapts to CF airways, but little is known of the selective pressures that drive this evolution. Through an extensive collection of 474 whole-genome sequences from clinical *P. aeruginosa* isolates, we have identified longitudinally sequential mutations in the RetS/GacA/GacS/RsmA regulatory system. This system reciprocally regulates genes responsible for directing the bacteria towards either acute or chronic lung infection pathways. RetS represses the effect of GacA, a transcriptional activator of the sRNAs, RsmY and RsmZ. These sRNAs, when expressed, sequester the transcriptional regulator, RsmA. RsmA activates expression of acute infection genes and represses transcription of chronic infection genes. The goal of the study is to gain insights into the adaptation of the isolates.
Methods: Using a global -omics approach, we have investigated the effects of these sequential mutations in eight isolates belonging to two clone types in order to characterize the resulting phenotypes associated with the different regulatory mutations.
Results: The first mutation occurs in retS, leading to a chronic infection phenotype. This mutation is followed up by a mutation in gacA or gacS, which may revert the effect of the retS mutation or lead to a new phenotype. Transcriptomics, proteomics, and metabolomics data will be presented to characterize the adaptational phenotypes.
Conclusion: The results of the investigation will be presented with the aim of understanding how the mutations may promote a progression of the infection by changing the regulation of the RetS–GacAS regulatory network caused by selective pressure of the CF airways.