97 Sputum colour and cultivation correlate in CF patients

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Changes of a sputum colour are believed to signal a bacterial infection of lower airways. The study analyses the relation between sputum colour and cultivation in all paediatric CF patients from South Moravia CF Centre in last 10 years.
944 sputum samples were analysed. Samples were obtained from 49 patients from 2000 to 2009 and cultivated. 382 samples had record of the sputum colour. Colours were divided into the 5 most prevalent groups: clear, white, yellow, green and brown. A correlation between the sputum colour and the cultivation of main CF lung pathogens was tested with Chi-square test.
Results from 944 samples: negative 33.8%, *Staphylococcus aureus* 29.7%, *Pseudomonas aeruginosa* 11.2%, *Haemophilus influenzae* 7.3%, streptococci 7.3%, *Burkholderia cepacia* type IV 2.4%, *Stenotrophomonas maltophilia* 1.6%, *Aspergillus* 0.5%, *Mycobacterium tuberculosis* 0.001%, contamination 8.6%. Sputum colours were: yellow 42%, clear 31%, green 14%, white 12%, brown 2%. Clear sputum correlates with negative cultivation ($p < 0.01$), 79% of clear samples were negative. White sputum is predominantly negative (55%), but without statistical significance. Yellow and green colour correlate with positive cultivation ($p < 0.01$). Only 6% of yellow and green samples were negative. Yellow samples yield: *S. aureus* 59%, *P. aeruginosa* 19%, *H. influenzae* 8%, *B. cepacia* 6%. Green sputum presents *S. aureus* 38%, *P. aeruginosa* 33%, *H. influenzae* 13%, *B. cepacia* 10%. Brown sputum yield: *P. aeruginosa* 43%, *S. aureus* 29%, *B. cepacia* 29%.
The study concludes that the clear sputum colour correlates with negative bacterial yield. White sputum is not specific. Yellow and green colour highly suggest the presence of *S. aureus* or *P. aeruginosa*. For *B. cepacia* green and brown is typical.

98* Evaluation of the MALDI-TOF system in the identification of non-fermenting Gram-negative rods recovered from sputum samples from cystic fibrosis patients

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Bacterial species recovered from respiratory samples of cystic fibrosis (CF) patients require different clinical management as they exhibit heterogeneous pathogenicity, thus their precise identification is important.
Objetive: We reassessed historical results obtained by WIDER (Fco. Soria Melguizo, Spain) and by API 20 NE (bioMérieux, France) using **MT** (MALDI BioTyper 2.0, Bruker Daltonik, Germany).
Methods: 266 isolates (65 *Achromobacter* spp., 56 *Burkholderia* spp., 52 *Pseudomonas aeruginosa, 20 Stenotrophomonas maltophilia,* 16 *Acinetobacter* spp., 10 non-*aeruginosa Pseudomonas,* 8 *Chryseobacterium* spp., 7 *Bordetella* spp., 4 *Ralstonia* spp. and 28 other non-fermenting Gram-negative rods) recovered from sputum of CF patients (1994–2009) and stored at −80ºC were re-identified using **MT**. Discrepancies were resolved with PCR of 16S rDNA and sequencing.
Results: 88% of isolates displayed identical identification at genus level by conventional methods and **MT** although it was only 68% at species level. Lack of identification (4.5%) with **MT** was ascribed to insufficient database entries. All *P. aeruginosa* were identified. *B. cepacia* complex (6) and *Burkholderia gladioli* (7), misclassified by routine methods, were correctly identified by **MT**. Additionally, *Pandoraea pnomenusa* (8) not previously identified by conventional methods in our laboratory were correctly identified by **MT**.
Conclusions: MT is a versatile tool that improves rapid identification of bacteria including those with limited biochemical reactivity frequently misidentified by the classical approach. Despite its accuracy, **MT** database needs to be enlarged mainly with infrequent species recovered from CF patients.

99* Contribution of mobile genetic elements to the competitiveness of a cystic fibrosis epidemic strain

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The Liverpool Epidemic Strain (LES) of Pseudomonas aeruginosa is a highly transmissible and successful cystic fibrosis (CF) pathogen and is associated with increased morbidity and mortality. The strain is prevalent across the UK and has been reported in North America. Recent sequencing of an LES isolate, LESB58, revealed the presence of multiple mobile genetic elements including five prophages, each of which can actively produce bacteriophages.
We have developed plaque assays for two of the novel phages and visualised them using electron microscopy. Signature tagged mutagenesis (STM) of LESB58 using a rat chronic lung infection model allowed identification of mutants attenuated in competitiveness. 162 mutants with reduced competitiveness in the model were identified, of which 12 had mutations within mobile genetic elements. For these 12 mutants we found no significant difference in production of exoproducts or resistance to six antibiotics in comparison to the wild-type. Six of the 12 mutants showed significantly reduced phage production and five could not produce one of the two novel phages. For some mutants growth characteristics in L-broth were affected: a mutation in a phage Clp protease led to an increase in growth rate and a mutation in a phage antitermination Q protein resulted in a dramatic reduction in bacterial numbers during stationary phase.
Our findings suggest that mobile genetic elements contribute to the competitiveness of the LES *in vivo* and that mutations in these regions affect both phage production and growth characteristics.
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100 Investigating the role of bacterial two-component systems in the microbiology of cystic fibrosis lung disease

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Two-component systems (TCSs) facilitate the adaptive response of bacteria to environmental stimuli. TCSs have been shown to play a pivotal role in the infectious process of many bacterial pathogens. As a result, TCS-deficient strains have been developed as live vaccines, whilst TCS-inhibitors are recognised as potential antimicrobial agents. The cystic fibrosis pathogens *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (Bcc) both harbour an exceptionally large number of TCSs. We aim to assess how the TCSs of both organisms contribute to virulence and/or antimicrobial resistance.
Our studies of *P. aeruginosa* are currently focused on the previously characterized PmrA/PmrB TCS, which is associated with resistance to colistin and related antimicrobials. We have identified several polymorphisms within the PmrA/PmrB proteins of colistin-resistant strains which may influence activity of the TCS and thus confer colistin resistance. Ongoing studies will assess the wider impact of these mutations on the organism's virulence and inflammatory properties. In contrast to the focused analysis on *P. aeruginosa* PmrA/PmrB, our studies of Bcc have involved a genome-wide analysis of previously uncharacterized TCSs. To date, we have constructed approximately 40 TCS-deficient *B. cenocepacia* (K56-2) mutants through targeted gene inactivation. Several of these TCS-deficient strains exhibit reduced virulence in the *Galleria mellonella* infection model and/or reduced biofilm formation. Future studies will aim to identify the genes that are regulated by these virulence-associated TCSs and investigate the activity of TCS-inhibitors as novel antimicrobials against these organisms.