Description of *Burkholderia contaminans* isolates recovered from sputum of cystic fibrosis (CF) patients with different courses of infection

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*B. contaminans* is the most frequent species of the *B. cepacia* complex (Bcc) isolated from CF patients in Argentina. There is little data on the virulence and clinical impact of this Bcc member over CF patients’ health.

To assess characteristics of *B. contaminans* associated with different courses of infection, colonies recovered from A) patients with a unique isolate (n=3) and B) first and last isolates of chronically infected patients (n=3), were compared. Two patients of this last group died.

RAPD genetic comparation, antibiotics resistance profile, virulence on the alfalfa model, biofilm formation, motility, haemolysis, green-yellow pigment and mucoid phenotype were studied.

Colonies recovered from A and first isolates from B were very similar and displayed haemolytic activity, pigment production, and mucoid expression. Among last isolates collected from the two dead patients, some colonies with nonmucoid phenotype, low motility and biofilm formation, no haemolytic activity nor pigment production were found. There were no significant differences of virulence in the alfalfa model assays.

Main differences were found in RAPD and the antibiotic resistance profiles: colonies collected from A group shared the same RAPD pattern and were susceptible to all antibiotics tested. However, only one colony of the first isolate from B group had this antibiotic profile. First and last isolates of the two dead patients showed genetic changes.

These results suggest genetic and phenotypic changes during the course of the infection by *B. contaminans*. Although a phenotype was detected at the last stage of infection, more virulence related to the alfalfa model could not be demonstrated.

Epidemiology and clinical impact of *Burkholderia cepacia* complex: A single-centre analysis

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Background: *Burkholderia cepacia* complex (Bcc), currently consisting of 17 species, is an important cause of morbidity and mortality in CF. *B. cenocepacia* and *B. multivorans* are the most commonly isolated species.

Method: Medical files of all CF patients ever infected with Bcc in the period 1998–2012 were reviewed. For each Bcc-infected patient, a control patient was matched for age, gender and genotype.

Results: Over this 14 year-period, 18 patients were infected with Bcc: 10 were chronically infected (8 *B. multivorans*, 1 *B. cenocepacia*, 1 *B. stabilis*) and 8 were transiently infected. Prevalence of Bcc varied annually with a maximum of 4%. Both groups, Bcc and control, had equal co-infection rates for *P. aeruginosa* (50 vs 40%, p=0.34) and *S. maltophilia* (11 vs 22%, p=0.37). However, in the group chronically infected with Bcc, none had co-infection with *S. maltophilia* (vs 50% in transient Bcc, p<0.5). In each group 1 patient died (1 *B. multivorans* and 1 control). Mean FEV1 1 and 2 years before and after acquisition was lower in Bcc than controls, but statistically not significant. At time of Bcc acquisition, FEV1 was significantly lower (p<0.3).

Conclusion: Prevalence of Bcc in our centre is stable and low. Bcc infections are predominantly caused by *B. multivorans*. *B. cepacia* infections did not occur. Bcc infection was not associated with a faster clinical deterioration. Lower lung function values at time of acquisition, suggest an association of Bcc acquisition and pulmonary exacerbation.

Direct detection, clonal analysis, and characterization of wild type and small colony variant strains of *Staphylococcus aureus* from the sputa of CF patients of the Prague CF centre

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*S. aureus* is a serious pathogen of CF patients. Altogether 1552 *S. aureus* isolates were recovered from 337 CF patients in 2009–2011. Isogenic strains of wild type and SCV *S. aureus* resistant to macrolides-lincosamides-streptogramin B (MLSβ) and/or aminoglycosides or MRSA were analyzed. Comparison of culture and PCR (duplex PCR – nuc, Sa2052) for the detection of *S. aureus* from the patient sputa revealed that as many as 40% of culture-negative samples were positive by PCR. The isolates were typed by pulsed-field gel electrophoresis (PFGE) and spa typing and some of them also by multilocus sequence typing. All isolates studied were of unique types. A small proportion of the MRSA isolates analyzed (n=3) showed an identical PFGE pattern and the same spa type. Around one third of the isolates were resistant to MLSβ antibiotics while aminoglycoside resistance was much less frequent. The isolates were screened for MLSβ resistance genes (ermA, ermB, ermC and msrA) and aminoglycoside resistance genes (ascA-aphB, aphaA3, adaC). In a large proportion (around one third) of the isolates, none of these resistance genes was detected. SCV strains, all but one, were also resistant to tetracycline and clindamycin. The highly sensitive duplex PCR could be a valuable tool for detecting early stage infection with *S. aureus* in infants or for assessing treatment efficacy in CF patients. *S. aureus* isolates from CF and non-CF patients may differ substantially in biological properties. Their role in the pathogenesis of the disease is still unclear.

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Staphylococcus aureus small-colony variants are independently associated with worse lung disease in children with cystic fibrosis

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Objectives: CF lung disease is associated with diverse bacteria chronically infecting the airways. Slow-growing, antibiotic-resistant mutants of *S. aureus* known as small-colony variants (SCVs) have been isolated from respiratory secretions from European adults and children with CF using specific but infrequently-used culture techniques. *S. aureus* SCVs can be selected either by exposure to specific antibiotics or by growth with *P. aeruginosa*. We sought to determine the prevalence, clinical significance, and likely mechanisms of selection of *S. aureus* SCVs, validation and extension of *S. aureus* SCVs, and evaluated associations with clinical characteristics using multivariable regression models.

Results: *S. aureus* SCV infection was detected among 24% of participants and was significantly associated with a much greater lung function decline during the study (p=0.007, adjusted for enrollment age and lung function), even after adjusting for infection with other known CF pathogens, including *P. aeruginosa* and methicillin-resistant *S. aureus*. Evidence indicated *S. aureus* SCVs were selected in *vivo* by treatment with trimethoprim-sulfamethoxazole and by co-infection with *P. aeruginosa*.

Conclusion: SCV *S. aureus* infection was independently associated with poorer CF respiratory outcomes in this pediatric cohort. As many clinical microbiology laboratories do not specifically detect *S. aureus* SCVs, validation and extension of these findings would require widespread changes in the usual laboratory and clinical approach to these bacteria.