Effect of *Staphylococcus aureus* supernatant on airway epithelium functionality in infants with cystic fibrosis

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*Staphylococcus aureus* colonizes very early the airways in CF patients. For a better understanding of the physiopathological mechanisms which are involved in the first stages of CF lung disease development, we analyse the airway epithelium from young asymptomatic CF infants. The aim of the present work was to study the effect of *Staphylococcus aureus* toxins on airway epithelium functionality.

Airway epithelial sheets collected from nasal brushings were analyzed for ciliary beating frequency measurement, for assessment of chloride and potassium efflux and for measurement of gap junction functionality.

In 13 CF infants diagnosed by neonatal screening and in 8 non-CF infants, nasal brushing was performed within 6 months after birth and incubated or not with *S. aureus* supernatant. Chloride efflux was significantly (p < 0.04) decreased in CF patients compared with non-CF patients. We also observed that *S. aureus* supernatant significantly decreased ciliary beating frequency (p < 0.02), chloride efflux (p < 0.01), voltage-dependent potassium efflux (p < 0.02) and gap junction functionality (p < 0.03) in non-CF and CF infants.

The present data demonstrate that, prior to any lung disease development in CF infants, toxins produced by *S. aureus* may alter the functionality of the airway epithelium and may favour the decline of pulmonary function in CF infants. Supported by Association Vaincre La Mucoviscidose.

Achromobacter xylosoxidans characteristics important for lung colonization of cystic fibrosis (CF) patients

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**Objectives:** *Achromobacter xylosoxidans* is an emerging pathogen increasingly isolated from respiratory samples of CF patients, often associated to antibiotic resistance. The present study was aimed to investigate virulent traits of 57 *A. xylosoxidans* strains isolated from CF patients and correlated the results with patients forced expiratory volume in 1 sec (FEV1%).

**Methods:** 57 *A. xylosoxidans* strains were isolated from sputum samples of CF patients attending at the Regional Centre of Cystic Fibrosis of the Umberto I’ Hospital of Rome. We carried out a genomic characterization by randomly amplified polymorphic DNA (RAPD) and evaluated *A. xylosoxidans* biofilm production on abiotic surfaces and antibiotic resistance. Resistance determinants were studied by Polimerase Chain Reaction (PCR) in highly resistant strains.

**Conclusion:** Our results demonstrate the ability of *A. xylosoxidans* to transfer its extended-spectrum β-lactamase-encoding plasmid. *A. xylosoxidans* strains had the specific band related to metal β-lactamase, and 3/7 of the them showed to possess specific band of the integron. All isolates showed to be biofilm producer and were divided in three classes. RAPD profiles resulted to be related to CF pathology status (estimated by FEV1% values). The ability to form biofilms and the multidrug resistance showed by *A. xylosoxidans* are certainly important factors influencing its virulence degree and seems to be bacterial features important for the colonization of CF patients with chronic infections and with more severe clinical conditions.

Characterisation of lipopolysaccharide from *Prevotella* spp. isolated from CF patients

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**Introduction and Objectives:** Bacterial lipopolysaccharide (LPS), a major component of the Gram-negative outer membrane, can elicit a potent response from the innate immune system and is recognised by various host cell receptors. The aim of this study was to characterise LPS from *CF* Prevotella isolates to provide insight into the potential role of *Prevotella* LPS in CF respiratory disease.

**Methods:** LPS from 48 clinical isolates, representing 15 distinct species and 2 *Prevotella* type strains were characterised. Belfast isolates (n=26), cultured from CF sputum, North Carolina isolates (n=7), cultured from sputum or BAL; UK non-CF invasive isolates (n=15). CF isolates were cultured from patients when clinically stable (n=29) or during pulmonary exacerbation (n=4). LPS was extracted using a micro-scale modification of the phenol hot water method, and characterised via Tricine SDS-PAGE and silver staining.

**Results:** LPS consisting solely of a lipid A-core oligosaccharide component. The absence of an outer O-antigen component may influence macrophage recognition of *Prevotella* spp. within the CF airways and result in a modified host immune response.

**Work funded by a Department of Employment and Learning, NI studentship to T. Matter and by HSC Research and Development, Public Health Agency, NI and the Medical Research Council through a US-Ireland Partnership Grant.**

Contamination of CF sputum with *Streptococcus constellatus*

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In CF sputum anaerobic conditions prevail. Along with *Pseudomonas aeruginosa* and obligate anaerobes, *S. constellatus* – a microaerophilic bacterium – can be found. *S. constellatus* is detected in high bacterial numbers in 39% of CF lung exacerbations. We investigated the interaction between *S. constellatus* and *P. aeruginosa* during anaerobic growth. *P. aeruginosa* strain PA01 was incubated anaerobically together with four *S. constellatus* patient strains diluted 10^-7 to 10^-5 fold. Colony forming units (CFU) were determined after three days. Lactate production was determined spectrophotometrically, the influence of lactate on *P. aeruginosa* growth was investigated. *P. aeruginosa* growth is reduced by one order of magnitude in the presence of all *S. constellatus* strains (2.9±1.5×10^5 vs 3.0±5.3×10^5 cfu/ml, p < 0.005). *S. constellatus* growth in the presence of *P. aeruginosa* is increased by one order of magnitude (4.5±3.2×10^7 vs. 7.7±2.5×10^8 cfu/ml, p < 0.001). All *S. constellatus* strains diluted 10^-7 fold could grow in a *P. aeruginosa* solution up to comparable bacterial numbers although the bacteria by themselves would not grow in this dilution. *S. constellatus* produces 22.0±3.2×10^8 cfu/ml lactate, but this amount of lactate does not influence *P. aeruginosa* growth. *S. constellatus* benefits from *P. aeruginosa* by reaching increased cfu. *P. aeruginosa* oxygen consumption may contribute to this fact. However, *S. constellatus* can also survive aerobically. Thus, other mechanisms must explain the growth advantage. Even minute amounts of *S. constellatus* successfully infect a *P. aeruginosa* solution explaining why *S. constellatus* easily may contaminate CF sputum.