

187 Ochrobactrum and Agrobacterium spp.: emerging opportunistic pathogens in cystic fibrosis patients?

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The genus *Ochrobactrum* and *Agrobacterium radiobacter* consist in Gram-negative rods. *Ochrobactrum* spp. are described as one of the most resistant Gram-negative rods. Although recognized as opportunistic pathogens, *Ochrobactrum* and *A. radiobacter* have received little attention during cystic fibrosis (CF) since one and four isolates are reported, respectively.

The aim of this study was to describe clinical and microbiological features of these bacteria in order to precise their clinical impact and their epidemiology in CF.

The strains were isolated during standard sputum analysis in CF patients; they were identified by both phenotypic and molecular methods and compared by pulsed-field gel electrophoresis (PFGE).

During a 4-year period, 16 *Ochrobactrum* and 10 *A. radiobacter* strains were isolated from 7 and 9 patients, respectively among the 200 patients analyzed. *Ochrobactrum anthropi* was cultured from samples of all the 7 patients together with *Ochrobactrum intermedium* (1 patient) or *Ochrobactrum pseudogrignonense* (1 patient). They were associated to other opportunistic pathogens or not. Two cases of chronic colonization by *O. anthropi* were proved by PFGE. Cross-contamination did not occur between patients. Clinical data were also reviewed.

Ochrobactrum spp. and *A. radiobacter* were more frequently isolated from respiratory tract during CF than in other patients. *O. intermedium* and *O. pseudogrignonense* were reported here for the first time in CF.

188 Large antibacterial spectrum of aminosterols derivatives towards multidrug resistant Gram-negative and Gram-positive bacteria from patients with cystic fibrosis

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Resistance to antibiotics is a life-threatening danger with more severe impacts on fragilized populations like cystic fibrosis (CF) patients. Squalamine and AminoS-terol Derivatives (ASDs) have demonstrated interesting antibacterial activity against bacterial reference strains. We provide herein the first report about the activity of squalamine 1 and two synthesized ASDs 2, 3 against 135 clinical strains of multidrug resistant Gram-negative and -positive bacteria. Further, we gained insight into their mechanism of action using Transmission Electron Microscopy (TEM). In the case of Gram-negative bacteria, MICs ranged from 2 to 128 mg/L. Mucoidity of *P. aeruginosa* strains and resistance to colistin significantly correlated with elevated MICs for tested compounds. In contrast, compounds 1–3 appeared very active against various Gram-positive bacteria with highest MIC value of 8 mg/L. TEM images revealed a membrane-disruptor effect of ASDs on *S. aureus* and altered membrane shape in treated *P. aeruginosa*. In spite of correlating with colistin in activity against Gram-negative bacteria, compounds 1–3 demonstrated surprising higher effect against Gram-positive isolates naturally resistant to colistin. Moreover, TEM images showed that ASDs affect differently the membrane of tested *S. aureus* and *P. aeruginosa* isolates. Taken together, our results indicate that ASDs possess a broad antibacterial spectrum with probably different mechanism of action against both Gram-negative and -positive bacteria. Further work is warranted to fully elucidate their mechanism of action and optimize their structure.

189 Human Neutrophil Peptide-1 (HNP-1) and a truncated analogue display antimicrobial activity against respiratory pathogens

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Malfunction of innate defense mechanisms and the lack of mucociliary clearance in the airways promote primary and recurrent bacterial infections in cystic fibrosis patients. Intensified usage of antibiotics in such patients has the adverse consequence of increasing antimicrobial resistance and alternative therapeutic interventions are urgently required. Antimicrobial cationic peptides are ubiquitous components of the innate immune system acting as the first line of defense against infectious agents and are interesting candidates for use in CF therapy. In the current study, a two-stage ultra sensitive radial diffusion assay was employed to determine the antimicrobial activity of both human neutrophil peptide-1 (HNP-1) and a truncated analogue (modelled on the C-terminal region; 2Abz23S29) against a panel of respiratory pathogens. A clinical isolate of MRSA, a major nosocomial pathogen with a progressive increased prevalence in CF populations was susceptible to both HNP-1 and its truncated analogue. Interestingly MRSA was more susceptible to both peptides (HNP-1 MIC value = 7.4 µg/ml; 2Abz23S29 MIC value = 36.5 µg/ml) than a clinical isolate of MSSA (HNP-1 MIC value = 18.4 µg/ml; 2Abz23S29 MIC value = 50.1 µg/ml). Clinical strains of *P. aeruginosa* displayed variable sensitivities to HNP-1 and its truncated analogue in line with their reported sensitivities to antibiotics. Members of the *B. cepacia* complex were resistant to both peptides. In conclusion, the finding that HNP-1 and a truncated analogue (which lacks disulphide bridges) display antimicrobial activity against MRSA, MSSA and *P. aeruginosa* could aid their potential use in therapeutic interventions in CF patients.

190 Serum tobramycin levels following delivery of tobramycin (TOBI®) via eFlow® advanced nebuliser in children with cystic fibrosis

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Background: Tobramycin (TOBI®) is widely used as a nebulised antibiotic in subjects with CF. Previous safety and toxicity data has mainly been performed using the Pari LC® Plus conventional nebuliser, yet many centres are increasingly using advanced membrane or mesh based nebulisers, such as the eFlow®.

Aims: To measure peak serum tobramycin levels in children aged 2–16 years using TOBI® via the eFlow®. To assess for renal and ototoxicity by measuring urinary NAG (N-acetyl-beta-D-glucosaminidase) and assessing annual audiology reports respectively.

Methods: 10 children attending Leeds CF Centre receiving 300 mg TOBI® via eFlow® for clinical reasons agreed to participate. Serum tobramycin levels were obtained one hour post nebulisation. Eight provided samples for urinary NAG, and nine underwent audiology.

Results: Mean age was 10.5 years (range 2 to 16). Serum tobramycin level was below 1 mg/L in 7 children, but the level was >1 mg/L in 3 children (maximum was 3.8). Two of the children with raised levels were 2 years old, the third was 11. Urine NAG/Creatinine levels were raised (>0.47 µmol/min/mmol) in 4 children, 1 of these had an elevated tobramycin level. Audiology results showed no change except in 1 patient who had high frequency hearing loss.

Discussion: Serum tobramycin levels over 1 mg/L can occur one hour post 300 mg TOBI® delivered by eFlow®. Raised urinary NAG levels suggest that some children may have some associated early renal toxicity. Further study is suggested to determine whether TOBI® dosage should be adjusted for age or weight of the paediatric patient when using eFlow®.