157* Ciprofloxacin shows concentration-dependent killing of *Pseudomonas aeruginosa* biofilm in vitro

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Background: Biofilm mode of growth is likely a main reason why the *Pseudomonas aeruginosa* (PA) lung infection in cystic fibrosis (CF) is rarely eradicated. The purpose of this study was to evaluate the killing activity of Ciprofloxacin on non-mucoid and mucoid PA biofilm on different time points *in vitro*.
Methods: Killing curves of Ciprofloxacin on biofilms of non-mucoid PAO1, PAO579, and mucoid PDO300 were made on days 1, 3 and 7. The microtiter plate method was used to test minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) for planktonic bacteria, and the minimal biofilm inhibition concentration (MBIC) and minimal biofilm eradication concentration (MBEC) were done on days 1, 3 and 7.
Results: Ciprofloxacin showed concentration-dependent killing activity for biofilms of non-mucoid PAO1, PAO579, and mucoid PDO300 on days 1, 3 and 7. The MIC and MBC for planktonic PAO1, PAO579, PDO300 were 0.125, 0.125, 0.25 μg/ml and 0.25, 0.5, 2 μg/ml respectively. MBIC of PAO1, PAO579, PDO300 was 1, 1, 4 μg/ml (day 1 biofilm); 4, 4, 8 μg/ml (day 3 biofilm) and 4, 8, 16 μg/ml (day 7 biofilm) respectively. MBEC of PAO1, PAO579, PDO300 was 16, 16, 32 μg/ml (day 1 biofilm); 64, 64, 128 μg/ml (day 3 biofilm) and 32, 64, 128 μg/ml (day 7 biofilm) respectively.
Conclusion: The killing activity of Ciprofloxacin was found to be concentration-dependent on biofilm growing PA of non-mucoid PAO1, PAO579, and mucoid PDO300. MBIC and MBEC of Ciprofloxacin were much higher than the MIC and MBC of the planktonic PA. MBIC and MBEC on day 3 and day 7 were higher than the value on day 1. The results suggest the importance of early treatment on biofilm infection of PA in CF patients.

158 Increasing antibiotic resistance with the Liverpool Epidemic *Pseudomonas aeruginosa* Strain (LES) – a 5-year study

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The use of powerful antibiotics therapy for infective exacerbations with Pseudomonas aeruginosa (Psa) in CF can induce antibiotic resistance over time, thereby complicating their treatment. Although infection with transmissible Psa strains has been shown to require more treatment and confer a worse prognosis, there have been no studies comparing the antibiotic resistance patterns of LES (the commonest UK transmissible strain, widespread in UK CF clinics) to unique Psa strains over time. To investigate this further, we matched (for age, years since CF diagnosis, FEV1, BMI, and Psa antibiograms to ciprofloxacin, tobramycin, ceftazidime, meropenem, tazocin and colomycin) 22 adult CF patients chronically infected with LES to 22 infected with unique strains and looked for changes in their Psa antibiograms over a 5 year period. Fisher's exact test was used to analyse statistical significance. LES became developed increasingly resistant antibiograms over time, compared to the unique strains (see table).

Antibiotic resistance in LES vs unique strains

	Year 1 Psa			Year 5 Psa		
	Unique	LES	p value	Unique	LES	p value
Sensitive	20 (90%)	17 (77%)	NS	19 (85%)	4 (18%)	<0.001
Multiresistant	2 (10%)	3 (13%)	NS	1 (5%)	14 (64%)	<0.001
Panresistant	0 (0%)	2 (10%)	NS	2 (10%)	4 (18%)	NS

For the first time, this study has shown that patients infected with the common transmissible UK Psa strain (LES) are becoming increasingly difficult to treat. This re-enforces the need to adopt effective cross infection control policies in the CF community to prevent spread of these damaging organisms.

159* Eradication of methicillin-resistant *Staphylococcus aureus* (MRSA) in adult patients attending a regional cystic fibrosis centre

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Introduction: This study evaluated the success rate of an eradication protocol for new isolates of MRSA in adults with CF attending a large Regional Adult CF Unit.
Methods: Case notes of all new MRSA isolates acquisitions between 1st January 2006 and 31st December 2009 were reviewed retrospectively. Patients with a new isolates of MRSA were offered eradication treatment [Unit protocol 2% nasal mupirocin, topical chlorhexidine 4% and nebulised vancomycin (250 mg twice daily) for five days, followed by three months of Rifampicin 600 mg daily and Sodium fusidate 500 mg twice daily]. From July 2007 these were prescribed for only one month.
Results: New isolates of MRSA were reported in 22 patients (median age 26 yrs, FEV_1 52% and BMI 22) with an incidence of 2% in 2006, 0.6% in 2007, 0.9% in 2008 and 2.8% in 2009. 21 patients received eradication therapy, time to treatment 10 days. Nasal/skin carriage was performed in 17 patients (81%), 2/17 were positive. Screening of family members was performed in 12 patients (57%), 1/12 were positive. To date 17 patients remain MRSA-free with a median follow-up of 13.5 months; success rate 81%. Four patients have regrown MRSA; median time to recurrence 15.3 (1.8–30.1) months; 2/4 had positive nasal carriage and/or positive family screening. Only one patient (4.8%) is chronically colonised. Increased success rate following change from three months to one month of oral antibiotic treatment (63% vs. 100%, $p < 0.001$).
Conclusions: Eradication of MRSA in patients with CF is feasible and has a high success rate. Determining carrier status and screening of family members is essential to identify those at risk of early recurrence.

160* Macrolide resistance of *Staphylococcus aureus* isolates obtained from respiratory tract samples of people with cystic fibrosis

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Introduction: The routine use of azithromycin in CF has been associated with increased macrolide resistance in *Staphylococcus aureus*. We have studied the phenotypic and molecular characteristics of this resistance in the Leeds CF clinics.
Methods: Single isolates of *S. aureus* were collected from respiratory samples of 84 people with CF between August 2007 and July 2008. MICs to erythromycin (ERY), clindamycin (CLI) and azithromycin (AZI) were determined by Etest. Resistance phenotypes were determined using ERY-CLI disc approximation. Presence of macrolide resistance genes *ermA, ermB, ermC* and *msrA* was determined by PCR. Clonal relationships between strains were determined using PFGE.
Results: Macrolide resistance was detected in 69/84 (82%) of isolates. Ten (12%) isolates were MRSA. AZI MICs ranged from 0.06 to >256 mg/l. Five different resistance phenotypes were seen using ERY-CLI disc approximation. In the macrolide-resistant isolates the genes most frequently detected were *ermC* (28% isolates) and *ermA* (26% isolates). PFGE showed that most isolates were clonally unrelated, except for a small cluster of a UK epidemic MRSA strain.
Conclusions: The recent rise in macrolide resistance in *S. aureus* isolated from people attending the Leeds CF clinics has not been caused by the dissemination of a resistant clone. Rather, the increase appears to have occurred through the multiple, independent emergence of a number of different resistance mechanisms in unrelated strains.