56 Antibiotic treatment of exacerbation of chronic airways infection
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Objectives: Repeated cycles of pulmonary exacerbation of chronic infection cause
tissue damage and progressive drop in lung function for CF patients so making
effective treatment and prevention strategies imperative. Our study aims to better
understand the response to antibiotics and identify biomarker targets suitable for
improved therapeutic or preventative treatments.

Methods: Multidimensional LC-MS/MS and relative quantitation was employed to
investigate the changes in sputum cellular proteome following antibiotic treatment
in 12 CF patients chronically infected with Pseudomonas aeruginosa.

Conclusions: 1989 human proteins were identified in total. 37 proteins out of
the 318 detected in >80% samples were differentially expressed (p < 0.05) after
antibiotic treatment. Of these, 22 exhibited a strong correlation (r = 0.969) be-
tween their pre-antibiotic/post-antibiotic ratios and ratios for the same proteins
comparing CF/healthy control cohorts, thus suggesting that successful treatment
promotes a proteome shift towards a non-CF profile. Ingenuity Pathway Analysis
software showed antibiotic treatment to most affect the molecular and cellular functions
of Cellular Movement (particularly Immune Cell Trafficking), Organismal Injury and Abnormalities, Cell Death and Free Radical Scavenging.
67 P. aeruginosa proteins and 17 proteins from other bacterial species were
also detected. Bacterial proteins accounted for 0.5–35% of CF sputum cellular
protein. Although total cellular protein (per g sputum) decreased following antibiotic
treatment, the percentage attributable to bacteria did not alter and no bacterial
proteins were detected as differentially expressed.

57 Azithromycin influence on biofilm formation of Pseudomonas
aeruginosa isolates from children with cystic fibrosis
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Growth of Pseudomonas aeruginosa in biofilm makes it more resistant to anti-
nicrobial treatment, and biofilm formation may reduce effectiveness of the antibiotic
therapy in children with cystic fibrosis (CF).

Aim: To study the biofilm formation ability of P. aeruginosa isolates from CF
children to optimize azithromycin (AZM) administration.

Materials and Methods: Formation of biofilms was studied by determination
the ability of 12 P. aeruginosa strains (6 mucoid and 6 non mucoid isolates) to adhesion
on the surface of 96-well polystyrole plate. Isolates were incubated in broth for 2 days
at 36.8°C, diluted 1/100 and inoculated in a 96-well polystyrole plate (8 replicates
for each isolate, in 4 replicates 5μg/ml AZM was added). Biofilms were grown for
2 days at 36.8°C in humid container, then planktonic cells were removed. Biofilms
were stained by 0.1% crystal violet, washed 3 times with distilled water. To estimate
biofilm formation, after 96% ethanol addition optical density (wave length 540 nm)
was measured by a plate-reader.

Results: Ability of biofilm formation was identified in 10 out of 12 isolates (83%),
2 isolates did not form biofilm. AZM suppressed biofilm development in 7 of 10
isolates decreasing biofilm density by 1.7–4 times. Suppression of biofilm for-
mation by other isolates was not significant.

Conclusion: In most cases, P. aeruginosa isolates from CF children were able
to form biofilm. Suppression of biofilm formation in 2/3 of examined P. aeruginosa
isolates induced by AZM confirms the need of further research in this field to
optimize macrolide antibiotics administration.

58 No antibiotic cross-resistance after 1 year of continuous
aztreonam for inhalation solution (AZLI) in cystic fibrosis (CF)
patients (pts) with chronic Burkholderia (BURK) infection
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Objectives: A 6 mo double-blind, placebo (PBO)-controlled trial of AZLI with 6 mo
open-label (OL) extension was conducted in CF pts chronically infected with BURK
to assess the effect of long-term continuous AZLI on BURK and P. aeruginosa (Pa)
spu tum density and antibiotic susceptibility (clinicaltrials.gov #NCT01059565).

Methods: Pts were randomized to 6 mo of daily AZLI 75 mg or PBO TID followed
by 6 mo of OL AZLI. Expectorated sputum or throat swabs were cultured for CF
pathogens and susceptibility testing of BURK and Pa isolates.

Results: No long-term suppressive effects on BURK and Pa sputum density were
observed in either study group. The BURK MIC50 of aztreonam and pip/tazo
increased 4-fold from baseline to the end of the randomized period in AZLI pts;
no other 4-fold changes were observed (Table). Pa isolates were more susceptible
than BURK isolates to all antibiotics.

One year of continuous AZLI did not compromise antibiotic susceptibility of BURK or Pa.
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59 Recommended doses of ceftazidime (CAZ) are insufficient to treat
less susceptible pathogens in cystic fibrosis (CF) patients
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Background: CF patients present altered b-lactams pharmacokinetics (PKs) and are
increasingly being infected by less susceptible strain of P. aeruginosa. The objective of this
study was therefore to assess the adequacy of recommended doses of ceftazidime (CAZ)
in this context.

Methods: We prospectively enrolled CF adults with acute pulmonary exacerbation
due to P. aeruginosa and treated with CAZ (200 mg/kg/day, in 3 injections, adapted
to renal function). PKs were calculated from CAZ concentrations measured by
HPLC-UV on serum before and 2 hours after the 30-min infusion. The clinical
breakpoint for P. aeruginosa of 8 mg/L, as defined by the European Committee
on Antimicrobial Susceptibility Testing (EUCAST), was used as target minimal
inhibitory concentration (MIC). CAZ therapy was defined as adequate if serum
concentration remained between 4 and 8 times the target MIC during at least 70% of
time.

Results: We measured the CAZ serum concentrations in 14 patients (8 males,
median age 31y (18–42), median BMI 20 (14–25) at day 2 (median, range 1–14)
of treatment. Only 3 patients (21%) had adequate concentrations, 7 (50%) had
insufficient and 4 (29%) excessive concentrations. Patients with insufficient con-
centrations tended to have higher creatinine clearance (122±23 ml/min, mean±SD)
than patients with overdosage (75±50 ml/min) (p = 0.15). No correlation was ob-
served between CAZ concentrations, plasma protein or BMI. All patients, but one,
reached PK targets for most sensitive strains.

Conclusion: Although recommended CAZ doses are sufficient to treat susceptible
P. aeruginosa strains, higher doses are probably needed for most patients in case
of less susceptible strains.