76 Antimicrobial activity of common essential oils toward cystic fibrosis respiratory pathogens

D.L. Chance[1], K.L. Mohr[2], A.K. Schwartz[2], J.P. Newman[2], T.P. Mawhinney[2,3]. [1]*University of Missouri, Molecular Microbiology & Immunology/Child Health, Columbia, United States;* [2]*University of Missouri, Experiment Station Chemical Laboratories, Columbia, United States;* [3]*University of Missouri, Biochemistry/Child Health, Columbia, United States*

Essential oils (EOs) have long been known to provide healing effects. This study's aim was to assess the inhibitory effects on cystic fibrosis (CF) pathogens of EOs commonly used in aroma and massage therapies for respiratory conditions. Disk diffusion (DD) and vapor exposure assays were employed, and EO zones of growth inhibition (ZOIs) recorded for agar-plated clinical isolates and laboratory strains of CF pathogens, including *P. aeruginosa* (PA), *S. aureus* (SA), and *C. albicans* (CA). A variety of common EOs inhibited mucoid & non-mucoid PA, SA, CA and other isolates. Sensitivities varied among isolates of a given species. Mixed cultures from sputa were generally more susceptible than those of throat origin. EOs with significant amounts of volatile components eugenol, eucalyptol, carvacrol, thymol, or cinnamaldehyde provided the widest range and most significant antibiotic activity, *i.e.* allspice, basil, bay, bay laurel, cassia, cinnamon, clove, oregano, and thyme white. MRSA were inhibited well in both diffusion and vapor assays by cassia, cinnamon bark, oregano, and thyme white oils (>25 mm ZOIs). Of other tested common oils used in rubs or inhalants, eucalyptus and wintergreen were most inhibitory. Oregano, thyme, and cassia oil were the most widely effective against mixed cultures obtained from polymicrobial CF specimens, (*i.e.* DD ZOIs ranges: 20–34, 22–31, 13–20 mm respectively). In conclusion, numerous essential oils were effective inhibitors of CF pathogens in vitro, suggesting that, if proven safe, such volatile oils (or modified components) may be useful as inhaled adjuvants to standard antibiotic therapies for chronic and/or complex cystic fibrosis infections.

77 Risk factor of multi-drug resistant *Pseudomonas aeruginosa* (MDR-PA) emergence in cystic fibrosis patients

M. Patout[1], S. Dominique[1], S. Pramil[1], L. Lemee[2]. [1]*Rouen University Hospital, Pneumology, Rouen, France;* [2]*Rouen University Hospital, Bacteriological Laboratory, Rouen, France*

Introduction: Infection with MDR-PA causes much concern among caregivers. MDR-PA is associated with a more rapid decline of FEV1 and more frequent antibiotic courses. The aim of this study was to identify risk factors for the emergence of MDR-PA defined by resistance to at least two of the following classes: β-lactamine, aminoside and quinolone.
Methods: Retrospective study including all the patients of our Cystic Fibrosis Department between 2008 and 2011 with at least one sputum culture showing PA. To compare patients with and without MDR-PA, clinical parameters and antibiotic courses before the onset of MDR-PA were analysed.
Results: Among 38 patients included, 14 had an infection with MDR-PA. The risk or protective factors for the emergence of MDR-PA determined in univariate analysis are detailed in Table 1 and Table 2 respectively.
Conclusion: MDR-PA emergence is associated with some patient's characteristics and more antibiotic use but also with the modality of antibiotic courses.

Table 1

	Odds ratio [95% CI]	p-value
Having a family member presenting cystic fibrosis	31.9 [6.9–147.3]	<0.001
Having a PA colonisation	7.1 [1.3–38.8]	0.007
Having a mucoid PA	5 [1.12–22.4]	0.029
Presenting a nasal polyposis	7.1 [1.6–31.3]	0.007
Receiving more frequently an antibiotic active against PA	12 [2.1–67.1]	0.002
Receiving more frequently an aminoside	7.1 [1.3–38.8]	0.015
Having a neutrophil range above median at exacerbation	8.6 [1.7–42.2]	0.02

Table 2

	Odds ratio [95% CI]	p-value
Receiving more frequently antibiotics by oral route	0.17 [0.04–0.71]	0.011
Receiving more frequently than median a mono antibiotherapy	0.15 [0.03–0.68]	0.01
Having a continous administration of Ceftazidim	0.17 [0.03–1.018]	0.045
Having a dosage of aminoside pic in sera more than median	0.06 [0.01–0.36]	0.001
Having a dosage of aminoside valley in sera more than median	0.09 [0.02–0.47]	0.003

78 Development of resistance in CF pathogens exposed to fosfomycin:tobramycin (4:1 w/w) under aerobic and anaerobic conditions

G. McCaughey[1], J.S. Elborn[1], M. McKevitt[2], M. Tunney[1], CF and Airways Microbiology Research Group. [1]*CF and Airways Microbiology Research Group, Queen's University Belfast, Belfast, United Kingdom;* [2]*Gilead Sciences Inc, Seattle, United States*

Background: Fosfomycin/tobramycin for inhalation (FTI) is being investigated as a treatment option for bacterial respiratory infection.
Aims: To investigate development of resistance when MRSA and *Pseudomonas aeruginosa* (*PA*) isolates from CF patients were exposed to fosfomycin, tobramycin and fosfomycin/tobramycin in a 4:1 (w/w) ratio (F:T) under aerobic and anaerobic conditions.
Methods: Late log phase cultures of MRSA (n = 5) and *PA* (n = 5) isolates were exposed to 2, 4 and 8 X aerobic and anaerobic MICs of fosfomycin, tobramycin and F:T. Spontaneous mutation frequency was calculated by dividing the number of resistant colonies after 48h by the original inoculum. Development of resistance after serial exposure was investigated by passaging *PA* (n = 3) and MRSA (n = 3) in sub-MIC concentrations of fosfomycin, tobramycin and F:T for 12 passages, with MICs determined every 3 passages.
Results: MRSA and *PA* isolates had lower mutation frequencies when exposed to F:T compared to fosfomycin and tobramycin alone under both conditions. In multi-step resistance studies, there was no increase in F:T MIC when *PA* and MRSA isolates were exposed to low concentrations of F:T for 12 passages. In contrast *PA* (n = 2) and MRSA (n = 3) isolates developed resistance to fosfomycin under aerobic conditions with one *PA* isolate also developing resistance under anaerobic conditions. One MRSA and 1 *PA* isolate developed resistance to tobramycin under aerobic conditions, while two MRSA isolates developed resistance under anaerobic conditions.
Conclusion: This study demonstrates that CF isolates are less likely to develop resistance when exposed to F:T compared to fosfomycin or tobramycin alone.

79 Molecular characterization of antibiotic resistance determinants in *Prevotella* species isolated from patients with cystic fibrosis

L. Sherrard[1], K. Graham[1], D.F. Gilpin[1], T. Schneiders[1], J.S. Elborn[1], M. Tunney[1]. [1]*Queen's University Belfast, CF & Airways Microbiology Research Group, Belfast, United Kingdom*

Background: Potentially pathogenic *Prevotella* spp. have been detected in sputum samples from CF patients. Standard treatment does not target such anaerobes as their role in disease progression is unknown.
Aims:
i. To determine *in vitro* antimicrobial susceptibility of CF and non-CF *Prevotella* isolates to antibiotics used in the treatment of both CF pulmonary infection and anaerobic infections and
ii. identify genes associated with resistance (*cfxA*/*cfxA2*, *nim* and *ermF*).
Methods: Susceptibility of *Prevotella* isolates from CF (n = 35) and non-CF (n = 49) patients, to amoxicillin, ceftazidime, clindamycin, co-amoxiclav, meropenem, metronidazole, piperacillin/tazobactam (pip/taz) and tobramycin was determined by E-test®. Each isolate was screened for *cfxA*/*cfxA2*, *nim* and *ermF* genes using PCR assays.
Results: Amoxicillin (55%), ceftazidime (29%) and metronidazole (4%) resistance was similar between CF and non-CF isolates. Clindamycin (CF, 58%; non-CF, 11%) and co-amoxiclav (CF, 26%; non-CF, 9%) resistance was higher among CF isolates. All isolates were sensitive to meropenem and pip/taz but resistant to tobramycin. Thirty of 45 (67%) isolates had reduced susceptibility to amoxicillin and were positive for *cfxA*/*cfxA2*. Eleven of 31 (35%) CF isolates were positive for *ermF* compared to 9/44 (20%) non-CF isolates. Three of 72 (4%) isolates were positive for *nim* but sensitive to metronidazole.
Conclusions: Amoxicillin resistance is common and can be linked with *cfxA*/*cfxA2*. Resistance to clindamycin (linked with *ermF*) and co-amoxiclav was more common among *Prevotella* isolates from CF patients. Metronidazole, meropenem and pip/taz resistance was not common.