

marrow-derived stem cells failed to migrate and differentiate. Whole cell patch-clamp and MEA recordings of grafted NPCs demonstrated that grafted cells developed properties of mature neurons and became functionally integrated into the host neuronal network.

Conclusions: In OHCs, grafted NPCs migrated to the pneumococcal induced area of hippocampal damage and differentiated into neurons that functionally integrated into the hippocampal network. The transplantation of neurosphere derived NPCs may hold promise for regenerative therapies aimed at repair of apoptotic brain damage in the hippocampus after BM.

O473 Host response during *Pseudomonas aeruginosa* adaptation to cystic fibrosis lung

C. Cigana, N.I. Lorè, S. Schwager, M. Juhas, L. Eberl, A. Bragonzi* (Milan, IT; Zurich, CH)

Objectives: Cystic fibrosis (CF) lung disease is characterized by transient airway *P. aeruginosa* infections and excessive neutrophil-dominated inflammation early in life followed by permanent chronic infection that causes persistent respiratory symptoms and a decline in lung functions. Here we aimed to dissect the host response to *P. aeruginosa* patho-adaptive strains during acute and chronic infection.

Methods: We analysed the pathogenicity of sequential clonal strains isolated from a CF patient during a period of up to 7.5 years in a multihost system including four different models, namely, *Caenorhabditis elegans*, *Galleria mellonella*, *Drosophila melanogaster* and mouse. In addition, the epithelial bronchial cells of CF origin IB3-1, their wt-like isogenic cells C38 and macrophage-like cells THP-1 were used to sustain the ability to provoke inflammation or damage during early or late phases of *P. aeruginosa* infection.

Results: *P. aeruginosa* strains at the onset of infection are more pathogenic than late isolates from the same patient when tested in *C. elegans*, *G. mellonella* and *D. melanogaster*. In murine model of acute infection, the early *P. aeruginosa* strain induced higher mortality than late clonal strains. Although attenuated in mortality, *P. aeruginosa* late isolates retained their capacity to persist in mouse models of chronic infection. H&E, PAS-staining and Tunnel assay of lung tissue sections showed that early strain induced pronounced leukocytes recruitment indicating strong inflammatory response while late strains increased numbers of mucin-positive goblet cells and apoptotic cells, a typical hallmark of damage in the airway chronic diseases.

To establish the "pathological drift", IB3-1 cells were infected with early and late *P. aeruginosa* clonal strains from CF patients and subjected to microarray analysis. Results indicated a decreased inflammatory response, including down-regulation of leukocyte receptors and adhesion molecules, and an increased damage mediated by tissue remodelling, due to late strains compared to early strains.

Conclusion: Our findings suggest that during long-term infection *P. aeruginosa* revises its interaction with CF host by activating alternative pathways including evasion of the immune response, non-inflammatory cell death and those relevant for tissue damage and remodelling process to ultimately result in chronic disease and decline in lung functions.

O474 In vitro and in vivo reduced fitness and virulence in ciprofloxacin-resistant *Acinetobacter baumannii*

Y. Smani*, R. López-Rojas, J. Domínguez-Herrera, F. Docobo-Pérez, S. Martí, J. Vila, J. Pachón (Seville, Barcelona, ES)

Objectives: Antimicrobial resistance confers a biological fitness cost on bacteria that may be manifested as a decreased growth rate, and reduced virulence. However, there are limited experimental data on the relative fitness and virulence of antimicrobial resistant *A. baumannii*. The aim of this study is to assess the fitness cost caused by ciprofloxacin resistant *A. baumannii*, as well as its virulence relative to the susceptible parental wild type (wt) strain in in vitro and in vivo murine peritoneal sepsis (MPS) model.

Methods: Two *A. baumannii* strains: 77wt (susceptible to ciprofloxacin) and its derivative 77R (resistant to ciprofloxacin) were used. Biofilm

formation was determined using crystal violet assay. Cellular viability of human lung epithelial cells (A549) was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Live/Dead[®], and lactate dehydrogenase (LDH) assays after addition of 77wt or 77R (10⁸ cfu/mL) for 24 h. Moreover, mortality and competition fitness between both strains was monitored in vitro and in vivo in a MPS model during 24 h.

Results: The acquisition of ciprofloxacin resistance by 77R strain reduces the formation of biofilm observed with the 77wt strain by 50%. A549 cells infected with 77R strain showed lower decrease in the cell death and release of LDH (86.53±2.44% and 5.98±1.81%) than 77wt strain (66.23±4.01% and 48.38±3.5%), respectively. Additionally, the assessment of A549 cells survival by Live/Dead[®] showed less dead cells, stained with ethidium homodimer-1 producing a bright red fluorescence, in A549 cells infected with 77R strain than with 77wt strain. Furthermore, resistance to ciprofloxacin acquired by 77R strain decreases the mortality of animals in MPS from 100% (observed with 77wt strain) to 20% at initial inoculum of 8.3 log cfu/mL. This resistance to ciprofloxacin slow the growth of 77R strain in vitro and in vivo MPS model in competition with the 77wt strain by 1.7 and 2.02 log at 24 h, respectively, compared to 77wt strain alone; thus, the in vitro and in vivo competition index were 0.02 and 0.03, respectively.

Conclusion: Our findings reveal the presence of an in vitro and in vivo fitness cost and reduced virulence of ciprofloxacin resistance in *A. baumannii*. The understanding of the gap between antibiotic resistance, biological fitness and virulence of *A. baumannii* will be useful to improve the antibiotic therapy against *A. baumannii*.

O475 Relationship between the gut microbiota and obesity in children and adolescents

V. Vankeerkhoven*, L. Bervoets, K. Van Hoorenbeeck, C. Lammens, S. Chapelle, C. Vael, K. Desager, H. Goossens (Antwerp, Hasselt, BE)

Objectives: Obesity is considered as one of the most important public health problems of our times. The last few decades the prevalence of obesity, especially among children and adolescents, has increased dramatically worldwide. The aim of our study was to determine whether the composition of the gut microbiota is related to obesity in childhood.

Methods: A cross-sectional study was set-up to examine the gut microbiota using faecal samples from 22 obese children and 33 non-obese children aged 6–16 years. The microbial composition in the faecal samples was analyzed by quantitative plating for *Staphylococcus* spp., *Bacteroides fragilis* group, *Clostridium* spp., *Lactobacillus* spp. and for *Bifidobacterium* spp.; matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identification of species of the *Bacteroides fragilis* group and quantitative real-time polymerase chain reaction (qRT-PCR) to determine the number of *Staphylococcus* spp., *Bacteroides-Prevotella-Porphyrionas* group, *Clostridium coccoides-Eubacterium rectale* group, *Clostridium leptum* subgroup, *Lactobacillus* spp., and *Bifidobacterium* spp. For statistical analysis, the BMI z-score was used as dependent variable thereby correcting for age and gender. A P-value of <0.05 was considered statistically significant.

Results: Both quantitative plating and qRT-PCR showed that the faecal concentration of the *Bacteroides fragilis* group in obese children was significantly lower than in non-obese children (P=0.017 and 0.018, respectively). Additionally, MALDI-TOF MS analysis demonstrated that obese children were colonized more frequently with *B. fragilis* than non-obese children (19.18% and 7.33%, P=0.039) whereas colonization with *B. vulgatus* was significantly higher in non-obese children compared to obese children (8.14% and 18.91%, P=0.016). Furthermore, *B. fragilis* was significantly positively correlated to the BMI z-score (P=0.03). Higher colonization with *B. fragilis* could therefore be associated with an increase in weight. The microbiota of obese children was also associated with a higher Firmicutes/*Bacteroides* ratio (P=0.02).

Conclusions: Significant differences were found in the composition of the fecal microbiota of obese and non-obese children. These results indicate that changes in the gut microbiota during childhood and

adolescence could lead to the development of obesity and that the gut microbiota could be an additional risk factor for obese-prone children.

Antifungals: in vitro and in vivo activity, pharmacokinetics and resistance

O476 EUCAST susceptibility testing of *Candida* species to echinocandins: improved separation between wild type isolates and fks mutants by supplementation of BSA to the test medium

M. Cavling Arendrup*, S. Park, J.L. Rodriguez-Tudela, W. Hope, C. Lass-Flörl, P. Donnelly, M. Cuenca-Estrella, D.S. Perlin (Copenhagen, DK; Newark, US; Madrid, ES; Manchester, UK; Innsbruck, AT; Nijmegen, NL)

Background: MICs of the three echinocandins against isolates with fks mutations from clinical failure cases are higher than those relative to wild type isolates, but the ranges of these susceptible and mutant populations either overlap or are just separated by 1–2 dilution steps. A recent preliminary study (Garcia-Effron, ICAAC M-352, 2009) reported that the addition of BSA to the growth medium leads to a better separation between wild type and mutant isolates using the CLSI method. We here investigated if this was also true for the EUCAST method.

Methods: MICs were determined for anidulafungin, caspofungin and micafungin by the EUCAST method without (EDef7.1) and with addition of 50 mg/mL BSA. A total of 94 clinical isolates including (no. of wt/no. of mutants) *C. albicans* (10/10), *C. glabrata* (9/11), *C. dubliniensis* (1/1), *C. krusei* (13/3), *C. parapsilosis* (19), and *C. tropicalis* (15/4) isolates. Three isolates harbour mutations outside the resistance hot spots and are regarded as wild type concerning echinocandin susceptibility because of their normal kinetic inhibition properties.

Results: The addition of BSA to the growth medium resulted in higher MICs for all isolates and all three compounds (Table). The increase was greatest for anidulafungin and micafungin, and notably greater for fks mutants compared to WT isolates. Among WT isolates the greatest increase was observed for *C. parapsilosis* which intrinsically harbours a "hot spot mutation".

Conclusion: Addition of BSA to the EUCAST growth medium enhances the MIC differences between fks hot spot mutants and wild-type isolates and thus increases the ability of the susceptibility test to differentiate between susceptible isolates and those harbouring resistance mutations.

Table. Increase in MIC₅₀ (in 2-fold dilution steps) for WT isolates and fks mutants using EUCAST supplemented with BSA compared to the EUCAST EDef 7.1.

	Anidulafungin		Caspofungin		Micafungin	
	WT	fks mutant	WT	fks mutant	WT	fks mutant
<i>C. albicans</i>	≥2	6	0	≥6	≥5	8
<i>C. dubliniensis</i>	4	≥7	2	≥4	≥7	≥6
<i>C. glabrata</i>	5	≥7	2	5	5	≥9
<i>C. krusei</i>	≥6	6	2	4	≥9	≥7
<i>C. parapsilosis</i>	≥6		4		≥8	
<i>C. tropicalis</i>	≥4	7	1	≥6	≥7	≥8

O477 Comparison of in vitro fungicidal activities of echinocandins against *C. albicans* in different peritoneal dialysis fluids

S. Tobudic*, H. Schranz, C. Kratzer, W. Poeppl, H. Burgmann (Vienna, AT)

Objectives: Continuous ambulatory peritoneal dialysis used in the treatment of patients with end-stage renal failure is often complicated by peritonitis. The current guidelines advocate the use of intermittent intraperitoneal (IP) antimicrobial management for peritoneal dialysis (PD)-associated fungal peritonitis. IP administration results in very high antimicrobial concentrations at the site of infection. Peritoneal dialyses fluids (PDFs) affect inhibitory efficacy on the microorganisms' growth, which may compromise the affectivity of some antibiotics. However,

there is no data about activity of antifungal agents in PDFs. The purpose of this study was to investigate in vitro the fungicidal effectiveness of three echinocandins in diverse PDFs.

Methods: The fungicidal efficacy of caspofungin, anidulafungin and micafungin against *C. albicans* ATCC 90029 was studied in the PDFs: Dianeal PD4® (glucose 1.36%, 2.27%, 3.86%), Physioneal 40® (glucose 1.36%, 2.27%, 3.86%), Extraneal® (7.5% icodextrin), and Nutrineal PD4® (1.1% amino acid) using time-kill curves. The protein concentration of the PDFs was adjusted to 2 g/l with human serum albumin (HSA) and the pH adjusted to 7.4 with NaOH, corresponding to conditions of used PDFs after a 4–6 h intraperitoneal dwell. Sabouraud bouillon (SAB) was used as a control broth. Ten milliliter of diverse PDFs and SAB containing yeast inoculum of approximately 106 CFU/ml was incubated for 2 h at 37 °C. Following incubation, the echinocandins at concentrations: 1×MIC, 4×MIC, 8×MIC were added. Samples were taken at 0, 2, 4, 6, 8, 10 and 24 h and the number of CFU/ml was determined.

Results: No difference in fungicidal activity between different echinocandins was shown. However, echinocandins were significant less active in PDFs than in control broth ($p < 0.01$) In SAB all three echinocandins at the concentration of 1×MIC attained 100% reduction of viable cells. In contrast, at concentration of 1×MIC echinocandins achieved no fungicidal activity (reduction <1 log₁₀ cfu/ml). At concentration of 4–8×MIC the highest decrease was detected in icodextrin containing PDF Extraneal®, however for 1.5 log₁₀ cfu/ml only.

Conclusion: Based on these in vitro data, we conclude that PD fluids using in clinical settings could impact the activity of echinocandins.

O478 Prospective surveillance of azole-resistance in *Aspergillus fumigatus* in the Netherlands

J.W. van der Linden, E. Snelders, G.A. Kampinga, B.J. Rijnders, E. Mattsson, Y.J. Debets-Ossenkopp, E. Kuijper, F.H. van Tiel, W. Melchers, P. Verweij* (Nijmegen, Groningen, Rotterdam, Utrecht, Amsterdam, Leiden, Maastricht, NL)

Objectives: Antifungal azoles are the cornerstone of the management of *Aspergillus* diseases. However, acquired azole resistance of aspergilli is increasingly reported, which compromises the use of this class of antifungals. The prevalence and spread of azole resistance in clinical *A. fumigatus* isolates is currently unknown.

Methods: We performed a prospective multicentre surveillance study to determine the prevalence of azole-resistance in *Aspergillus* species. Between June 2007 and January 2009 medical microbiology laboratories of 7 Dutch University Medical Centres screened all clinical *Aspergillus* isolates for resistance to itraconazole (ITZ) using Sabouraud agar-slants supplemented with 4 mg/l of ITZ. Phenotypic susceptibility profile and the mechanisms of resistance were determined for resistant isolates. Patient characteristics were also collected.

Results: In total 2,062 *Aspergillus* isolates from 1,385 patients were screened of which 87% were identified as *A. fumigatus*. 82 isolates were ITZ-resistant, of which 79.8% was also resistant to voriconazole and 16.7% to posaconazole. In 90.2% of ITZ-resistant *A. fumigatus* isolates a L98H substitution combined with a 34 base pair duplication in the Cyp51A-gene promoter region was found. The overall prevalence of ITZ-resistance in *A. fumigatus* was 5.3% (range 0.8 to 9.5%). Patients with a hematologic/oncologic disease were more likely to harbour an azole-resistant isolate compared to patients groups with other underlying diseases ($p = 0.02$). 64% of patients in whom a resistant isolate was recovered were azole-naïve and the mortality-rate of patients with azole-resistant invasive aspergillosis was 88%.

Conclusion: Multi-azole-resistance in *A. fumigatus* is wide-spread in the Netherlands and is associated with a poor outcome in patients with invasive aspergillosis.