***Background.*** Cryptococcosis is an opportunistic fungal infection caused by both *Cryptococcus neoformans* and its sibling species, *Cryptococcus gattii*. Flucytosine (5FC) is one of the most widely used antifungals against *Cryptococcus* spp., yet very few studies have looked at the molecular mechanisms responsible for 5FC resistance in this pathogen.

***Methods.*** Eleven *Cryptococcus gattii* clinical isolates were selected based on differential 5FC susceptibility. All isolates underwent whole-genome sequencing and genomic differences in key genes involved in flucytosine metabolism were examined. Heterologous expression of FCY1 and spot sensitivity assays were performed to examine regions of interest based on genomic differences.

***Results.*** Susceptibility assays and sequencing analysis revealed an association between a point mutation in cytosine deaminase (FCY1) and 5FC resistance in two *C. gattii* clinical isolates, B9322 and JS5. This mutation results in the replacement of arginine for histidine at position 29 and occurs within an unconserved stretch of amino acids. Heterologous expression of FCY1 and spot sensitivity assays demonstrate that the point mutation did not have any effect on FCY1 activities and was not responsible for 5FC resistance. Comparative sequence analysis further show that no amino acid changes were observed in either cytosine permeases (FCY2-4) or uracil phosphoribosyltransferase (UPRTase, encoded by FUR1) among 5FC resistant and 5FC susceptible *C. gattii* isolates.

***Conclusion.*** Together, our work suggests that the mediator(s) of 5FC resistance in B9322 and JS5 is likely found either downstream of FUR1 or on disparate regulatory pathways that modulate flucytosine metabolism. These findings suggest clinical 5FC resistance in *C. gattii* may occur by a nontraditional mechanism(s).

***Disclosures.*** **All authors:** No reported disclosures.

### 277. Fungal Mechanobiology: High Shear Forces Increase *Rhizopus* Virulence

Alexander Tatara, PhD[1]; Nathaniel Albert, MS[2] and Dimitrios P. Kontoyiannis, MD, ScD, PhD (Hon), FACP, FIDSA, FECMM, FAAM[3]; [1]Medicine, Baylor College of Medicine, Houston, Texas, [2]MD Anderson Cancer Center, Houston, Texas, [3]The University of Texas MD Anderson Cancer Center, Houston, Texas

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***Background.*** It has been observed in both civilian and military populations that high-energy events, such as tornados and blast injuries, have been associated with mucormycosis in otherwise immunocompetent patients. However, the effects of high shear force directly on fungal biology have not been explored. In order to elucidate the relationship between fungal mechanobiology and virulence, *R. oryzae* was exposed to high shear stress. Subsequent changes in virulence were measured in a validated fly model of mucormycosis. Finally, spores were simultaneously exposed to high shear forces and calcineurin inhibitors to determine whether this classical stress pathway was involved in changes in virulence in response to shear force.

***Methods.*** $10^4$ or $10^7$ spores/ml of *R. oryzae* in 100 ml saline were either: (1) grown in static culture (CNTRL); (2) subjected to stirring at 1100 RPMs for 30–45 minutes (Tornadic Physical Shear Challenge, TPSC), or 3) subjected to TPSC in the presence of the calcineurin inhibitor tacrolimus (TPSCS + TAC). Wild-type flies were subsequently infected via dorsal thorax inoculation and monitored for survival over 7 days ($n = 26$ per group; performed in triplicate).

***Results.*** Flies inoculated with *R. oryzae* exposed to high shear stress experienced significantly greater mortality compared with spores grown under static conditions ($P < 0.001$). Co-culture of spores grown under TPSC with tacrolimus (1 mg/ml) resulted in increased fly survival ($P < 0.001$). In fact, there was no significant difference between flies inoculated with spores subjected to high shear and TAC and spores grown under static conditions ($P = 0.934$).

***Conclusion.*** Fungal exposure to high shear stress increases virulence. As calcineurin inhibition completely mitigated the effect of shear stress on Mucorales virulence, activation of the calcineurin stress response may play an important role in the mechanotransduction of shear stress to increased fungal virulence.

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### 278. Optimization of the CRISPR/Cas9 System to Manipulate Gene Function in *Rhizopus delemar*

Clara Baldin, PhD[1]; Sameh Soliman, PhD[2]; Heewon Jeon, BS[1]; Christopher Skory, PhD[3]; John Edwards, MD[1,4] and Ashraf Ibrahim, PhD[1,4]; [1]Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California, [2]Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharjah, United Arab Emirates, [3]Agricultural Research Services, USDA, Peoria, Illinois, [4]David Geffen School of Medicine at UCLA, Los Angeles, California

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***Background.*** The genus *Rhizopus* is the main cause of mucormycosis, a life-threatening infection that affects predominantly hosts with an impaired immune system. However, patients with severe trauma and burns, without prior immune deficiency, are also at increased risk of developing mucormycosis. Despite aggressive treatment that involves disfiguring surgery and antifungal therapy, mortality rates range from ~50 to 100%. Genetic manipulation of *Rhizopus* is critical for identifying fungal targets to develop more effective therapies. However, *Rhizopus* genetics are challenging because of lack of dominant selection markers, low efficiency of transformation, and rarity of chromosomal integration. Here we attempted to adapt the CRISPR/Cas9 technology to disrupt genes in *R. delemar*.

***Methods.*** We used the Gibson cloning strategy to assemble all necessary elements of the CRISPR/Cas9 system in one plasmid using the *pyrF* as a selection marker. The targeted gene for disruption was a toxin-encoding gene with similarity to ricin. This disruption cassette was transformed using biolistic delivery system into *R. delemar* pyrF strain (M16). Recombination events were studied by Southern blot analysis and ricin gene expression was analyzed by qRT-PCR. Furthermore, damage to alveolar epithelial cells (A549) and nasal epithelial cells (CCL30) was studied with $^{51}$Cr-release assay.

***Results.*** Five stable transformants were obtained with the CRISPR/Cas9 construct. Southern blot analysis and nucleotide sequencing confirmed a partial deletion of the ricin gene, in the region where the guide RNA was designed. Moreover, gene disruption was confirmed by abrogation of ricin expression in comparison to reference strains (wild type or mutant with the CRISPR/Cas9 plasmid void of ricin gene sequence). Finally, ricin-mutants showed significant reduction in damage to A549 cells and CCL30 cells when compared with the reference strains (20–30% reduction, $P < 0.01$ by $t$-test).

***Conclusion.*** We have successfully adapted the CRISPR/Cas9 system to disrupt the ricin-like gene in *R. delemar.* This tool will enable us to better understand the pathogenesis of mucormycosis and ultimately aid in designing novel and more successful strategies to manage this lethal fungal infection.

***Disclosures.*** **All authors:** No reported disclosures.

### 279. Studies of *Pseudomonas aeruginosa* Mutants Indicate Pyoverdine as the Central Factor in Inhibition of *Aspergillus fumigatus* Biofilm

Gabriele Sass, PhD[1]; Hasan Nazik, PhD[1]; John Penner, MD[2]; Hemi Shah, BS[1]; Karl V. Clemons, PhD[3]; Marie-Christine Groleau, MSc[4]; Eric Déziel, PhD[5] and David A. Stevens, MD, FIDSA[3]; [1]California Institute for Medical Research, San Jose, California, [2]Stanford University School of Medicine, Stanford, California, [3]Stanford University, Stanford, California, [4]INRS-Institut Armand-Frappier, Laval, QC, Canada, [5]INRS-Institut Armand Frappier, Laval, QC, Canada

**Session:** 48. Fungus Among Us: Basic Science
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***Background.*** *Pseudomonas aeruginosa* (Pa) and *Aspergillus fumigatus* (Af) are common opportunistic bacterial and fungal pathogens, respectively. They often co-exist in airways of immunocompromised patients and individuals with cystic fibrosis, where they form biofilms and cause acute and chronic illnesses. Hence, the interactions between them have long been of interest, and known that Pa can inhibit Af in vitro.

***Methods.*** We have approached the definition of the inhibitory Pa molecules by studying 24 Pa mutants, with various genes deleted, for their ability to inhibit Af biofilms. The ability of Pa cells, or their extracellular products produced during planktonic or biofilm growth, to affect Af biofilm formation, preformed Af biofilm, or planktonic Af was studied in agar and liquid assays with conidia or hyphae.

***Results.*** Four mutants, *pvdD-/pchE-*, *pvdD-*, *lasR-/rhlR-*, and *lasR* were shown defective in the various assays. This suggested a central place for the siderophore pyoverdine as the key inhibitory molecule, although additional quorum sensing-regulated factors likely contribute to the deficiency of the latter two mutants. Studies of pure pyoverdine substantiated these results and restored inhibition by pyoverdine deletion mutants. Added iron or hemin reversed the inhibition by pyoverdine or pyoverdine-producing Pa strains.

***Conclusion.*** The key inhibitor for Af biofilms produced by Pa is pyoverdine. The inhibitory mechanism is chelation of iron, and denial of iron to Af.

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### 280. *Aedes aegypti* and *Aedes albopictus* Ocurrences in Guatemala

Manuel Lepe-Lopez, VETT[1]; Miriam Canet, MD[2]; Marcela Davila, VETT[1]; Edson Flores, VETT[3]; Yaimie Lopez, VETT[4]; Amilcar Davila, VETT[1] and Luis Escobar, VETT[5]; [1]Departamento de Investigación y Extensión, Facultad de Medicina Veterinaria y Zootecnia, Universidad de San Carlos de Guatemala., Guatemala, Guatemala, [2]Infectious Diseases, INorth CarolinaAN, Guatemala, Guatemala, [3]Instituto de Ciencia Animal y Ecosalud, Facultad de Medicina Veterinaria y Zootecnia, Universidad de San Carlos de Guatemala., Guatemala, Guatemala, [4]Centro de Estudios en Salud, Universidad del Valle de Guatemala., Guatemala, Guatemala, [5]Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota, USA, Guatemala, Guatemala

**Session:** 49. Global Health Potpourri
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***Background.*** Recent emergence of zika and chikungunya along with the continuous prevalence of dengue in Guatemala has become a threat to public health resulting in high morbidity and mortality. According to national epidemiologic vigilance reports, the prevalence for dengue, chikungunya, and zika are 53.42, 30.96, and 19.02 per 100,000 habitants respectively. Despite cases of the diseases being reported countrywide, the regional occurrences of *Aedes aegypti* and *Aedes albopictus* are unknown, with some studies reporting the presence of the vectors in only four of 22 departments.

***Methods.*** National active larval entomologic surveillance information was obtained and the results where validated through Geographical Information Systems tools to generate a map of occurrences of either *Aedes aegypti* or *Aedes albopictus*. The information was compared with the reports of cases of the diseases associated with these vectors.