**S8.3d**
**Characterization of glycosylphosphatidylinositol-linked aspartyl proteases in *Candida glabrata*: Role in pathogenicity**

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S8.3 How the Fungal Cell Wall Glycan Can Modulate the Immune Response?, September 23, 2022, 3:00 PM - 4:30 PM

*Candida glabrata* is the second to fourth most common yeast pathogen found in *Candida* bloodstream infections, depending upon the geographical location. *C. glabrata*, which belongs to the Nakaseomyces clade, possesses a distinct set of virulence attributes which include the ability to survive and proliferate in macrophages, adhere to biotic and abiotic surfaces and survive a wide range of stresses. Our research is focused on unveiling the strategies that *C. glabrata* employs to survive the nutrient-poor hostile host environment and evade host immune response. Toward this end, we are delineating the cellular processes, that are regulated by the family of 11 putative glycosylphosphatidylinositol-linked, cell surface-associated aspartyl proteases (CgYapsins, CgYps1-11). We have recently characterized the secretome of *C. glabrata* wild-type and aspartyl protease-deficient mutant strains, and showed that the GPI-anchored aspartyl proteases are both bonafide constituents and key modulators of the *C. glabrata* secretome. Further, besides elucidating the role of CgYapsins in the suppression of the host pro-inflammatory immune response, we have identified the flavodoxin-like protein CgPst2 as a substrate of the CgYps1 protease and demonstrated that the CgYps1-mediated cleavage of CgPst2 is pivotal to oligomerization and activity, and functions of CgPst2 in quinone detoxification. These findings underscoring the importance of multifunctional CgYapsins in the physiology and pathogenesis of *C. glabrata* will be presented.

**S8.4b**
**Population biology of hedgehog fungus *Trichophyton e r inacei***

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S8.4 Cases of animal mycoses, September 23, 2022, 3:00 PM - 4:30 PM

*Trichophyton erinacei* is a main cause of dermatophytosis in hedgehogs and is increasingly reported from human infections worldwide. It is found in wild European hedgehogs (*Erinaceus europaeus*) but also in the African four-toed hedgehog (*Atelerix albiventris*), a popular pet animal worldwide. Little is known about the taxonomy and population genetics of this pathogen despite its increasing importance in clinical practice. Notably, whether there are different populations or even cryptic species associated with different hosts or geographic regions is not known. To answer these questions, we collected 161 isolates, performed phylogenetic and population-genetic analyses, determined mating type, and characterized morphology and physiology. Multigene phylogeny and microsatellite analysis supported *T. erinacei* as a monophyletic species, in contrast to highly incongruent single-gene phylogenies. Two main subpopulations, one specific mainly to *Atelerix* and the second to *Erinaceus* hosts, were identified inside *T. erinacei*, and slight differences in the size of microconidia and antifungal susceptibilities were observed among them. Although the process of speciation into two lineages is ongoing in *T. erinacei*, there is still gene flow between these populations. Thus, we present *T. erinacei* as a single species, with notable intraspecies variability in genotype and phenotype. The data from wild hedgehogs indicated that sexual reproduction in *T. erinacei* and *de novo* infection of hedgehogs from soil are probably rare events and that clonal horizontal spread strongly dominates. The molecular typing approach used in this study represents a suitable tool for further epidemiological surveillance of this emerging pathogen in both animals and humans. The results of this study also highlighted the need to use a multigene phylogeny ideally in combination with other independent molecular markers to understand the species boundaries of dermatophytes.

**S8.5c**
**MLST genotyping and phylogenetics of AD-hybrids**

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S8.5 Genotyping of *Cryptococcus neoformans* and *C. gattii*, September 23, 2022, 3:00 PM - 4:30 PM

Objectives: In a previous study a set of new molecular-type specific primers were designed to apply the standard ISHAM consensus multi-locus sequence typing (MLST) scheme to *Cryptococcus neoformans* AD hybrids. In the present study, we report the preliminary results of the investigation by MLST of a large number of AD hybrids with the aim to identify the circulating genotypes, their phylogenesis, and population genetics.

Methods: A total of 50 AD-hybrid isolates from different parts of the world and from different sources were genotyped by MLST. Minimum spanning trees using GoeBurst algorithm were generated by comparing hybrid genotypes and by comparing separately either allele-A and allele-D portions of the hybrid genotypes to the haplotypes recorded in the MLST global database.

Results: Analysis identified 32 hybrid genotypes grouped in three distinct main clusters (CC12, CC21, and CC30) including 12 isolates each. Both CC12 and CC21 clusters included isolates from different countries and continents but the former grouped only isolates with mating type aADalpha whereas the latter those with mating type alphaADa. Cluster CC30 included only isolates from Ivory Coasts. Heterozygous allelic combinations in each of the seven MLST loci presented two or three combinations more frequent than the other ones. In some isolates, one or more alleles were not amplified after multiple attempts, and therefore, they were considered as lacking. A total of 22 MLST profiles were identified by analyzing separately the allele-A combinations of the hybrids. Comparison with all MLST profiles of VNI, VNII, and VNB included in the MLST global database showed that the allele-A portion of the hybrid genotypes was grouped in few VNI or VNB clusters. In none of the investigated hybrids, the allele-A portion originated from VNII genotypes. Similarly, when the MLST profile of allele-D portion of hybrids was compared to all VNIV genotypes present in the global MLST database, few clusters were identified but, in this case, mostly originated from genotypes not yet found among VNIV haplotypes.

Conclusions: These preliminary results suggest that the AD hybrids here investigated originated from the mating of A haploids very common in both clinical and environmental isolates and D haploids that are not circulating at present or are very rare. Therefore, it is likely that hybrids originated in the environment where VNIV genotypic diversity is higher and suitable AD combinations can occur. Sequencing of further AD hybrids is in progress to confirm these results.

**S8.5d**
***Cryptococcus neoformans* and *Cryptococcus gattii* clinical isolates from Colombia develop heteroresistance to fluconazole at high concentrations**

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S8.5 Genotyping of *Cryptococcus neoformans* and *C. gattii*, September 23, 2022, 3:00 PM - 4:30 PM

Introduction: Cryptococcosis is a worldwide mycosis caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. Although resistance to antifungals is infrequent, isolates with decreased susceptibility to fluconazole have been reported globally, including Colombia, which may be due to 1) heteroresistance, defined as the ability to adapt to increasing concentrations of this azolic antifungal, and 2) point mutations in the EGR11 gene encoding the fluconazole target enzyme, lanosterol 14-$\alpha$-demethylase.

Objective: To determine the development of heteroresistance to fluconazole in *C. neoformans* and *C. gattii* clinical isolates from Colombia and to amplify and sequence the ERG11 gene of the isolates to seek for mutations that might characterize resistant or heteroresistant phenotypes.

Methods: The minimum inhibitory concentration (MIC) to fluconazole was determined in 31 and 24 isolates of *C. neoformans* and *C. gattii*, respectively, using broth microdilution. Heteroresistance was evaluated by plating each isolate on YPD agar that contained fluconazole at concentrations equal to the MIC of each isolate. Heteroresistant colonies were then replated at increasing concentrations of fluconazole, as high as 128 $\mu$g/ml.

Results: All isolates were susceptible to fluconazole with MICs of 1 $\mu$g/ml ($n = 3$), 2 $\mu$g/ml ($n = 6$), 4 $\mu$g/ml ($n = 17$), 8 $\mu$g/ml ($n = 23$), 16 $\mu$g/ml ($n = 5$), and 32 $\mu$g/ml ($n = 1$). However, all isolates developed heteroresistant colonies, with increments in the MIC from 2 to 6 dilutions. Notably, 5 (16.1%) isolates of *C. neoformans* and 8 (33.3%) of *C. gattii*, grew up to 64 $\mu$g/ml of fluconazole, which is the MIC that defines resistance to this azole, and 1 (3,2%) isolate of *C. neoformans* and 4 (16.7%) of *C. gattii* grew up to 128 $\mu$g/ml of fluconazole. Currently, the ERG11 gene is being amplified for further sequencing. Conclusion: clinical isolates of *C. neoformans* and *C. gattii* that develop heteroresistance to fluconazole in high concentrations circulate in Colombia, which is important since this characteristic contributes to the relapse of cryptococcosis during therapy with this triazole.

**S9.1d**
**Chronic pulmonary aspergillosis in post-TB and retreatment TB patients in Lagos, Nigeria**

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S9.1 Chronic Pulmonary Aspergillosis - where do we stand?, September 23, 2022, 4:45 PM - 6:15 PM

Objectives: Chronic pulmonary aspergillosis (CPA) is a known complication of post-TB treatment. It is a progressive disease characterized by progressive cavitation, fibrosis, and pleural thickening among others. Globally, an estimated 3 million people are affected. This study determined the burden of CPA amongst the post-TB and retreatment TB patients in two facilities in Lagos, Nigeria.

Methods: This was a prospective longitudinal study that was carried out at two TB clinics (LUTH and NIMR) in Lagos, Nigeria between February 2021 and March 2022. The study cohorts were patients that had been previously managed (2-4 years earlier) for TB, they were clinically classified as retreatment TB and post-TB patients. Patients were seen in clinics every 3 months and the following data were collected: Quality of life (WHO and SGRO questionnaires used), 5 mls of blood for *Aspergillus* IgG level (using Bordier; cut-off of 0.8 AU/ML), sputum for culture (those with productive cough), and chest X-ray. Infectious disease society of America (IDSA) case definition was used to determine cases of CPA.

Results: A total of 112 post-TB treatment patients were recruited, 60 (53.1%) were retreatment TB and 52 (46.0%) post-TB patients. The mean age was 41.14 years; with the majority between the ages of 21-30 years. The male/female ratio was 0.9/1. 98 (87.5%) were HIV negative, and only 40 patients had GeneXpert testing done. In all 32/40 were GeneXpert negative; of which 24/32 and 8/32 belonged to the retreatment, and post-TB groups respectively. Cough was the predominant symptom with 39 (34.8%) having productive cough. Hemoptysis occurred in 11 (9.8%), 10 in the retreatment group with 2 having frank hemoptysis. Chest imaging revealed that 27/112 of the studied cohort presented with multiple cavities, 4/112 had single cavities, 26/112 had cavities with surrounding opacities and 23/112 had upper lobe consolidation. A total of 17/112 of them had bilateral lung infiltrates and 13/112 had pleural thickening. Sputum culture yielded growth of *Aspergillus* spp, with *A. flavus* (*n*-11; 36.7%) being the predominant species followed by A. *fumigatus* (*n*-10; 33.3%), and *A. niger* (*n*-9; 30%). In all 38/112 (33.93%) patients had *Aspergillus* IgG titer above the cut-off level, while 6 symptomatic patients had borderline *Aspergillus* IgG levels. A total of 11/112 (9.82%) of the study cohorts with positive *Aspergillus* IgG levels were also culture positive; 8/68 of the *Aspergillus* IgG negative patients were culture positive and had abnormal chest imaging reported. A total of 38 (33.93%) were confirmed cases of CPA using IDSA criteria. Of the GeneXpert positive; 7/40 were retreatment TB; 16/32 of GeneXpert negative and 8/40 of GeneXpert positive met the criteria for CPA.

Conclusion: Our findings demonstrate that CPA is easily misdiagnosed as treatment failure TB or TB relapse. There is a need for further follow-up of post-TB patients for early identification of post-TB lung disease. It is also imperative to educate our clinicians to screen patients who have persistent symptoms and are GeneXpert negative for other post-TB lung diseases.

**S9.2d**
**Directed evolution of voriconazole resistance in *Aspergillus fumigatus* identifies novel mutations responsible for triazole resistance**

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S9.2 Azole resistance in *Aspergillus fumiagatus*: how hot is your hotspot?, September 23, 2022, 4:45 PM - 6:15 PM

*Aspergillus fumigatus* is the leading invasive mold pathogens in humans. The first line of treatment for invasive *A. fumigatus* infections are the triazole antifungals that inhibit Erg11/Cyp51 lanosterol demethylase activity, blocking ergosterol biosynthesis.

In recent years, triazole resistance of *A. fumigatus* has been increasingly reported, both as a result of widespread agricultural use of fungicidal triazoles and long-term treatment in patients with chronic aspergillosis. To date, the most common triazole resistance mechanisms in *A. fumigatus* are alterations in the erg11A/cyp51A gene or promoter, followed by overexpression of efflux pumps and mutations in hmg1, encoding HMG-CoA reductase. To identify novel triazole resistance mechanisms, we passaged *A. fumigatus* wild type and cyp51A-null strains under increasing concentrations of voriconazole (0.25 $\mu$g/ml-20 $\mu$g/ml) to generate resistant strains. Resistant isolates were whole-genome sequenced and compared with untreated controls. We identified known cyp51A and cyp51B mutations, and novel mutations in HMG1 and in previously uncharacterized genes in the ergosterol biosynthesis pathway as well as several efflux pumps. We identified at which stage of evolution each of the mutations occurred as well as their contribution to the resistance phenotype by re-introduction, alone and in combination, into the susceptible parental strain. Our study identified novel genes conferring triazole resistance and helps outline the complex stepwise evolutionary paths by which *A. fumigatus* develops resistance.

**S9.3d**
**Antivirulence drug discovery to disarm *Candida albicans* with metabolites from myxobacteria.**

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S9.3 Drug resistance in emerging pathogenic fungi, September 23, 2022, 4:45 PM - 6:15 PM

*Candida albicans* is an opportunistic fungal pathogen. While nearly 40%-60% of humans are colonized harmlessly by *C. albicans*, prolonged use of antibiotics or an immune-compromised status can lead to mucosal or deadly systemic candidiasis (Kumamoto et al., 2020). There are only three classes of antifungals available to physicians to treat and prevent severe Candidiasis. These drugs, which include polyenes, echinocandins, and azoles, protect the host by killing or inhibiting growth of *C. albicans*, thereby exerting a strong selective pressure toward drug resistance.

Antivirulence drugs, by contrast, target virulence traits of pathogens without affecting their growth, making drug resistance less likely to evolve. We seek to discover novel antivirulence compounds against *C. albicans* that keep it at, or return it to, its baseline commensal state even under conditions that support pathogenicity.

Bacteria have evolved a large repository of metabolites that can inhibit and modulate the behavior of other organisms in their environment. Clades like the predatory myxobacteria are untapped for potential antifungal and antivirulence drug

discovery (Vij et al., 2021). We here screened ≈ 2800 crude extracts from myxobacteria in an *in situ C. albicans*-mammalian epithelial cell infection model. Typically, when oral epithelial cells (TR146) are infected by *C. albicans*, the fungus proliferates, forms hyphae, and invades and damages the monolayer. In our assay, we estimate the damage to epithelial cells by released lactate dehydrogenase. We also note changes to the growth and morphology of the fungus. Based on these readouts we assign antivirulence and antifungal ranks to each extract, and confirm top-ranked hits with an independent propidium iodide-based assay for host cell damage, and a colorimetric assay of fungal metabolic activity. We found that several of the top-ranked antifungal extracts also showed effects on a multi-drug resistant strain of *C. auris*.

Using an established pipeline, we identified several of the antifungal and antivirulent bioactive components in these extracts. After scaling the production of promising lead compounds, we will test their drugability on clinical *Candida* spp. strains, and identify their mode of action using large-scale *Candida* spp. knock-out libraries and multi-omics approaches.

Our tested extracts are likely to contain new classes of non-toxic antifungals that can potentially treat infections by multi-drug resistant fungi. We identified and confirmed several myxobacteria extracts that protected mammalian epithelial cells without severely affecting the fungus' growth, which are, therefore, considered antivirulent.

Sources:


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**S9.4a**

**Ocular infections by melanized fungi *Curvularia lunata* and *Lasiodiplodia theobromae*: Antifungal susceptibility and clinical outcome**


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S9.4 Free oral presentations (late breaking), September 23, 2022, 4:45 PM - 6:15 PM


Purpose: To report antifungal susceptibility and clinical correlations in melanized fungal isolates of *Curvularia lunata* and *Lasiodiplodia theobromae* from ocular infections.

Methods: Antifungal susceptibility testing was performed by broth microdilution testing, following Clinical and Laboratory Standard Institute guidelines, of 17 *C. lunata* and 13 *L. theobromae* isolates from monomicrobial infections of microbial keratitis or fungal endophthalmitis patients. Isolates resistant to ≥2 classes of antifungals were considered as multidrug-resistant (MDR). The panel of antifungals tested were amphotericin B, natamycin, voriconazole, ketoconazole, fluconazole, itraconazole, posaconazole, and caspofungin.

Results: Voriconazole showed the highest susceptibility (83.3% isolates) followed by natamycin (80%), fluconazole (80%), itraconazole (76.7%), ketoconazole (70%), posaconazole, and caspofungin (66.7% each) and lastly amphotericin B (63.3%). For treatment, all patients received topical natamycin, and few received additional oral ketoconazole or intraocular voriconazole. MDR isolates led to the poorer clinical outcomes ($P$=.015) in patients. But natamycin resistance alone did not show unfavorable outcomes ($P$=.28), though this was the most frequent drug used topically in fungal ocular infections.

Conclusion: Melanized fungi causing ocular infections have varying susceptibility to different antifungal agents. Most effective drug as seen *in vitro* in our study, was voriconazole. Significant resistance to amphotericin B, which is the most common antifungal used in intravitreal injections, was noted. MDR isolates overall, had poorer clinical outcomes.