(R-WTM) was significantly higher (p < 0.05) than those with mutation (R-WTM) and the sensitive isolates (1.2-11 v 0.2-2.5, and 0.3-2.2, respectively). Although the R-WTM and R-L_WTM background (p < 0.05) CD2 and MBI expression compared to S isolates, noticeable variation was not seen among the other genes. Protein homology modeling and molecular docking revealed that the mutations in the ERG11 gene were responsible for structural alteration and low binding efficiency between ERG1p and ligands. Isolates with ERG11 mutations also possessed ASDDC in ERG11 and together T0305, G711A mutations in CYP2C.

Conclusions: Nonmutant mutations in the ERG11 gene and coordinated overexpression of various genes including different transporters, ergosterol biosynthetic pathway, transcription factors, and stress-responsive genes are associated withazole resistance in clinical isolates of C. tropicalis.  

5.3a Unravelling the genetic determinants of virulence in Cryptococcus neoformans

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5.3.1 Cellular phenoloxidase and fungal virulence, September 22, 2022, 3:00 PM - 3:30 PM

Cryptococcus neoformans is a human pathogenic basidiomycete yeast that can cause cryptococcal meningitis (CM), predominantly in immunocompromised individuals. The patient outcome depends on both host and pathogen-specific factors, including C. neoformans genetics. A groundbreaking study 2012 was the first to show that patient outcome is associated with genetic differences between C. neoformans isolates. Subpopulation-wide-predictive sequencing studies have revealed over 150 sequence types (ST) of C. neoformans that are associated with both geographic location and clinical outcome. All these studies have been broad, examining the severity of disease cryptococcal phenotypes in a collection of highly diverse strains. We chose a narrow focus and collected various genotypic and phenotypic data from a single ST. ST53 is a common sequence type isolated from patients globally and is the most common clinical isolate found in the sub-Saharan African country of Uganda. Previously, we performed whole genome sequencing on 34 ST51 Ugandan clinical isolates. We identified 652 unique SNPs in this ST53 population compared to the H99 reference genome. We also showed that ST53 contained two subpopulations: ST51A and ST51B. In the current study, we further characterized the genotypic, phenotypic, and virulence differences between these 38 clinical isolates. Using Illumina sequence data, we identified a pattern of linkage disassociation that suggested that ST51A and ST51B are evolving separately. We performed long-read sequencing on each isolate to investigate chromosomal changes and large somatic variations in 15 of the cases to identify a chromosomal rearrangement event within strains of ST51, which had recombinated with chromosome 3. Additionally, we characterized several in vitro phenotypes for each isolate and identified three distinct phenotypic clusters based on cell wall and capsule content and growth experiments. Next, we inferred which ST53 isolates and observed eight different disease manifestations, including isolates that caused non-CNS infections. Overall, by working within a single sequence type, we can gain a deeper understanding of how some small genetic changes can impact strain-specific phenotypes while others have no discernible effect. Eventually, these data can be used to provide valuable information about how each clinical isolate impacts patient outcomes.  

5.3b fungal species: Initiators of colonization and infection

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5.3.2 Cellular phenoloxidase and fungal virulence, September 22, 2022, 3:00 PM - 3:30 PM

Pungo produce asialo and asialo-ses for reproduction and distribution, which can be both in space and time. Distribution in space occurs, by air movement, and also by water or other vectors such as litter organisms. Fungal fungi from the division Ascomycota that belong to the order Enteromycetales produce asialo-ses called conidia. Conidia are considered stress-tolerant cells and are also able to survive unfavorable conditions such as thermal stress, dehydration, osmotic pressure, cold stress, variation in pH, and UV. For example, conidia of the fungus Fusarium oxysporum are isolated worldwide and are often regarded as a cause of death because conidia easily 'fall' from the location of production, but are still able to make the higher altitudes. There is indirect evidence that spores may also be able to transport large distances in the air. For example, the fungus Syncephalis conidia have been detected in the air over thousands of kilometers from the Saharan Desert to the Caribbean seas.  

Distributions in time is occurring as stress-tolerant cells remain dormant at one location for an extended period, surviving conditions that are more favorable for growth. Some ascospores (sexual spores) are extremely stress-tolerant and dormant for very long periods. Other species extend dormancy in a dry state. When microbial species are inherently variable, stress resistance varies between strains from the same species. For example, conidia heat resistance (KRF) of various strains of the fungus Paracoccidioides var. ranged between 5 to 5.7°C for 24 h. This intraspecific variation could have profound consequences on diagnostics, virulence, and antifungal treatment in clinical settings.

The fungicide germination, the presence of nutrients such as inorganic salts, sugars, and amino acids is required. The swelling phase of conidia is also called sporulation growth. Swollen conidia direct the growth to one side of the cell to grow in a polarized fashion, which leads to the formation of a growth pole (polarized growth). There is a notable drop in stress resistance during isotropic and polarized growth and genes expressed during these stages might represent novel targets for fungal infection.

5.3c Investigating the link between phenoloxidase and virulence in Cryptococcus neoformans

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5.3.3 Cellular phenoloxidase and fungal virulence, September 22, 2022, 3:00 PM - 3:30 PM

Objectives: Fungal pathogens Cryptococcus neoformans and Cryptococcus gattii are responsible for hundreds of thousands of annual deaths in immunocompromised individuals. Considerable phenotypic variation is exhibited by strains in response to stress encountered during host infection, including increased capsule and cell size, the release of shed capsule, and the production of giant (≥ 15 µm), micro- (<1 µm), and irregular cells. We aimed to investigate whether the production of melanin is correlated with virulence using two sets of strains. The first is a collection of diverse clinical isolates obtained from HIV/AIDS patients in Botswana with accompanying clinical data. The second is a collection of lines derived from the C. neoformans type strain H99 with high genetic similarity but differing levels of virulence. Some linesages in this set possess a mutation in SGC2, which encodes a component of the SAGA histone acetyltransferase complex that has previously been implicated in their virulence.

Methods: Isolates were cultured under conditions that stimulate stress encountered in vivo (DMEM, 5% C02, 37°C) and these were known to induce capsule production and induce cell wall changes. Cells were counted with indoxyl, visualized by light microscopy, and phenotypes were scored. For clinical isolates, MLST analysis was performed to determine their relationships. For H99 strains, Cadmium-n-mercaptoethanol assays, growth curves, and antifungal susceptibility testing was performed to confirm their intrinsic virulence and growth profile. Serial block face and regular scanning electron microscopy were used to investigate the intracellular morphology of the giant, micro, and irregular cells to confirm that they possess attributes of fungal cells.

Results: Substantial phenoloxidase was seen across both collections. In the clinical strain set, phenotypic variations fall into two groups associated with differing symptoms. The production of large phenoloxidase was associated with a higher C02 count and was negatively correlated with intracellular process indicators, suggesting that these are induced in early-stage infection. Small phenoloxidase were associated with lower C02 counts, negatively correlated with mucin production indicators, and positively correlated with intracellular process indicators, suggesting that they are produced later during infection and may promote proliferation and dissemination. Isolates possessing giant cells, microcells, and shed capsules were rare, but strikingly, we associated them with patient death.

In the H99 set, strains from HIV/AIDS patients had larger average capsule size, greater variation in cell size, and increased production of microcells and shed capsules. Deletion of SGC2 in an intermediate virulence lineage substantially increased its production of microcells and released capsules, consistent with a switch to hypervirulence. SGC2 knock-out mutant was subsequently identified in clinical isolates and was found to be significantly correlated with patient death. Expansion of a TA repeat in the second intron of SGC2 in clinical isolates was posteriorly correlated with cell capsule size, suggesting that it also affects SGC2 function.

Conclusion: Our results extend the evidence for a link between phenoloxidase and virulence, with a likely role in ergopeptide metabolism mediated by SAGA-induced histone acetylation.
Cutaneous manifestations of deep fungal infections: A retrospective study from one tertiary hospital

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Objective: Analysis of the cutaneous manifestations in patients with deep fungal infections to provide a basis for clinical differentiation and diagnosis.

Methods: Patients who presented to our hospital from 2016 to 2021, were definitively diagnosed with deep fungal infections by histopathology and mycological detection. Isolates of focal infections were cultured in vitro on SDA or MDA media for 14 days and the species were identified by morphological or molecular analysis. Relevant clinical data on epidemiology, skin manifestations, underlying disease, causative fungal agent, treatment, and outcomes were collected and analyzed.

Results: A total of 15 patients were diagnosed with deep fungal infections. The respiratory system (7/15) was the most easily involved primary focus of deep fungal infection, digestive system (3/15) and nervous system (2/15) were less common. The mean age of the patients was 50.30 years. Of these, 8 were males. More than half of the cases (7/15) were presented in immunosuppressed patients, including long-term glucocorticoid use, organ transplantation, tuberculosis infection, and malignancy. Skin manifestations were varied, with plaques (5/15) being the most common type of lesion, and then papules (4/15), nodules (2/15), patches (2/15), and ulcers (2/15). Candida spp. (9/15) was the most common pathogens, followed by Talaromyces marneffei (2/15) (Fig. 1a), Cryptococcus spp. (2/15) (Fig. 1b), and Aspergillus spp. (2/15). One case had co-infection with C. albicans and Aspergillus spp.

Conclusions: Patients with deep fungal infections are often accompanied by skin manifestations, which vary between patients with deep fungal infections caused by different pathogenic fungi.