S5.4d

Agrochemicals potentiate multidrug resistance and alter virulence in *Cryptococcus neoformans* via hitch-hiking effect of aneuploidy

Liuliu Sun[1,2], Yong-bing Cao[3], Tian-hua Yan[2], Yuan-ying Jiang[1], Feng Yang[1]
[1]Department of Pharmacy, Shanghai Tenth People's Hospital, School of Medicine, Tongji University, Shanghai, China
[2]Department of Physiology and Pharmacology, School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing, China
[3]Department of Vascular Disease, Shanghai TCM-Integrated Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China

S5.4 Free oral paper session, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: *Cryptococcus neoformans* is an environmental fungal pathogen causing fatal pulmonary and disseminated infections in humans, especially in AIDS patients. However, only three antifungal drugs are available for the treatment of cryptococcosis: fluconazole, amphotericin B, and flucytosine. *C. neoformans* is worldwide distributed in nature, but environmental factors influencing antifungal drug susceptibility are largely unknown.

Methods: We tested the antifungal effect of agrochemicals against *C. neoformans* lab strain H99. We obtained drug-resistant adaptors. We sequenced the adaptors and got a list of adaptors bearing unique aneuploidy. We asked if aneuploidy caused multidrug resistance to antifungal drugs and if aneuploidy altered virulence *in vitro* and *in vivo*. We also investigated the molecular mechanism of aneuploidy-mediated multidrug resistance.

Results: Here we found tebuconazole and benomyl, belonging to two distinct classes of agrochemicals, could inhibit the growth of H99. However, H99 could gain resistance to both drugs mainly via aneuploidy. Importantly, particular aneuploidy also caused multidrug resistance to fluconazole, amphotericin B, and flucytosine. Furthermore, aneuploidy also altered virulence *in vitro* and *in vivo*. We found copy number variation of genes on the aneuploid chromosome caused a proportional increase in transcript and protein levels.

Conclusion: Therefore, we posit agrochemicals could enhance multidrug resistance and alter the virulence of *C. neoformans* via the hitch-hiking effect of aneuploidy.

S5.5a

Standing up FungiNet: Genomic epidemiology and surveillance of fungal diseases

Nancy Chow[1], Lindsay Parnell[1], Patricia Escandon[2], Nelesh Govender[3], Tom Chiller[1]
[1]Centers For Disease Control and Prevention, Atlanta, United States
[2]Instituto Nacional de Salud, Bogota, Colombia
[3]National Institute for Communicable Diseases, Johannesburg, South Africa

S5.5 Genomic Epidemiology of Fungal Infections, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: With the advent of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) sequencing, the public health landscape for genomic epidemiology and surveillance has transformed for a variety of pathogens. For fungal diseases, the U.S. Centers for Disease Control and Prevention (CDC) is working with global partners to stand up FungiNet, a network that aims to equip scientists with laboratory, bioinformatics, and informatics resources to harness genomic data. FungiNet partners will use genomic and epidemiologic data to detect outbreaks, identify introductions, and characterize transmission of fungal infections. In 2022, FungiNet aims to onboard nine state and local health departments in the United States and two global partners, the Instituto Nacional de Salud in Colombia and the National Institute for Communicable Diseases in South Africa, with a focus on *Candida auris*.

Methods: To streamline the onboarding process, CDC generated standardized operating procedures (SOPs) specific to *C. auris*. For DNA extraction, SOPs were created for workflows using the Zymo Research Quick-DNA™ (ZR) Fungal/Bacterial Miniprep, Qiagen Dneasy Blood and Tissue, and Epicentre (Illumina) MasterPure Yeast DNA Purification kits. For library preparation and Illumina sequencing, PulseNet methods used for foodborne pathogens were validated for *C. auris*. For NCBI data submissions, required data elements were defined. For SNP and phylogenetic analyses, the bioinformatics workflow MycoSNP was adapted to use Nextflow software and the Terra platform. For visualization with epidemiologic data, guidance documents and tutorials for Microreact were created. Finally, for data reporting, processes are being designed in REDCap and in laboratory information management systems to rapidly share genomic-related data.

Results: To date, 11 partners have committed to building capacity for *C. auris* genomic sequencing and analysis as a FungiNet partner. Of these, seven have validated methods for DNA extraction, and nine have generated high-quality sequencing data. Only one partner has installed and locally run MycoSNP, and none have submitted raw sequence data to NCBI.

Conclusions: Currently, 11 FungiNet partners are working to onboard *C. auris* genomic sequencing and bioinformatics analysis in 2022. This process is complex, requiring several laboratories, bioinformatics, and informatics workflows. For many partners, bioinformatics analysis and NCBI submission are the most challenging activities with the installation of MycoSNP and the ability to batch upload data to NCBI as the main barriers. Next steps will focus on the validation of informatics methods to link genomic and epidemiologic data.

S5.5d

SARS-CoV-2 associated invasive fungal sinus infections; the Sri Lankan perspective

H Thabrew[2], Liyanage Shamithra Madhumali Sigera[1], A P Anand[3], R A D M Ramanayake[3], Munasinghe M V Munasinghe M V[3], W L L N Wickramasekara[3], P I Jayasekera[3]
[1]Manchester Fungal Infection Group, Core Technology Facility, University of Manchester, Manchester, United Kingdom
[2]Department of Microbiology, University of Karapitiya, Galle, Sri Lanka
[3]Department of Mycology, Medical Research Institute, Colombo, Sri Lanka

S5.5 Genomic epidemiology of fungal infections, September 22, 2022, 3:00 PM - 4:30 PM

Introduction: The spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to 663 426 reported cases and 16 506 deaths as of May 5, 2022, in Sri Lanka recording the worst pandemic of the modern era. Coronavirus 2019 (COVID-19) associated mucormycosis has caused an epidemic within the pandemic in the neighboring India leading to soaring case numbers estimated at 140 per million population recording 80 times higher prevalence in comparison to developed countries. On the contrary, Sri Lanka has not seen a rise in COVID-19-associated fungal infections on a similar scale, even though geophysical conditions are shared.

Objective: This study was done with the aim of exploring the epidemiology of COVID-19-associated invasive sinus infections in Sri Lanka.

Methods: A retrospective study was done on the sinus samples received from patients suspected of having COVID-19-associated invasive fungal sinusitis. The study was done during the third wave of the pandemic in Sri Lanka from May 1, 2021 to March 31, 2022. Multiple sinus samples from all SARS-CoV-2 PCR-positive patients received at the Mycology Reference Laboratory of the Medical Research Institute were included in the study. The presence of *pauci septate* broad fungal filaments with wide angle branching was considered suggestive of mucormycosis and the presence of mucormycetes fungi in the culture of the specimen was considered confirmatory for mucormycosis. Similarly, the presence of thin hyaline branching septate fungal filaments was considered suggestive of fungal infection, and the organism was confirmed by the presence of growth in the culture. Available details on the request forms were analyzed to identify demographic data and risk factors in COVID-19-associated invasive sinus infections.

Results: A total of 133 sinus samples were received from 102 SARS-CoV-2 PCR-positive patients during the third wave. All 45 patients (44%, 45/102) had positive findings indicating fungal sinusitis. The median age was 56 (IQR 48-61) years in the patients with fungal sinusitis. A majority (60%) of them were female patients. Microscopic and/or culture-positive mucormycosis was demonstrated in 35 (34%, 35/102) patients. Culture-proven mucormycosis was seen in 20 patients while direct microscopic evidence was seen in 28 patients. All mucormycosis patients isolated *Rhizopus arrhizus* which was identified by morphological methods. A total of 4 patients (4%, 4/102) had *A. fumigatus* growing in the culture, 2 patients with *A. flavus* and 1 with *A. terreus* sinus infection were seen. Mixed growth of *A. flavus* and *Rhizopus arrhizus* was seen in 1 patient. Risk factors were not mentioned in 23 patients. Diabetes mellitus was found in 31% (11/35) of patients with mucormycosis, while 2 patients had chronic kidney disease and 1 had hypertension.

Conclusion: This study demonstrates that fungal sinusitis is a significant entity in Sri Lanka with 34% proven mucormycosis infection in the samples received at the Mycology Reference Laboratory from SARS-CoV-2 PCR positive patients. Diabetes mellitus was seen in 31% of the COVID-19-associated mucormycosis cases. However, further studies are required to establish the effect of risk factors on mucormycosis.

S6.1d

First case report of pediatric blood stream infection by *Candida magnoliae* in a known case of B cell ALL post -induction chemotherapy in Central India

Subhasish Bhadra, Karuna Tadepalli, Narendra Kumar Chaudhary, Arati Bhadade
All India Institute of Medical Sciences, Bhopal, Bhopal, India

S6.1 Antifungal Prophylaxis in Children with Cancer and HSCT, September 22, 2022, 4:45 PM - 6:15 PM

Objectives: Documentation and dissemination of findings of a rare fungal isolate in an immunosuppressed child.

Methods: A case study with rare fungal isolate in correlation to age, clinical condition, sample, and comorbidity was done. A 6-year-old male child was admitted for routine management of B cell acute lymphoid leukemia. The patient completed induction chemotherapy in July 2021. The patient was planned for consolidation in the last week of July and to rule out any infection blood and urine samples were sent. Paired blood samples were received in pediatric automated BacT/Alert blood culture bottles. After 8 days both the blood culture bottles flashed positive. On gram stain, budding yeast cells oval to globose were seen. No pseudohyphae were seen. Nigrosin staining result was negative. It was processed further on HiCrome™ agar showing cream-colored colonies at 370 C, cornmeal agar with 1% tween 80 for Dalmau technique showed only oval to globose yeast cells with blastoconidia, enlarged cells appearing as chlamydoconidia without pseudohyphae or true hyphae were seen. Glucose and sucrose were fermented and trehalose was weakly fermented. Urease was negative. Isolate was identified as *Candida glabrata*/*Candida auris*. Antifungal susceptibility showed elevated MIC for fluconazole but susceptible to amphotericin B, voriconazole, and caspofungin. As part of routine collaboration with reference center PGIMER, bloodstream *Candida* isolates were sent for confirmation, and quality control.

Results: The isolate phenotypically suspected as *C. glabrata* causing fungemia was confirmed by the reference center as *C. magnoliae*. Currently, patient is on routine follow-up and doing well. On reviewing of available literature on *C. magnoliae*; bloodstream infections in two low birth weight neonates from Brazil, one immunocompetent child with tenosynovitis from the USA, and a terminal oncology patient from Italy were noted.

In a Chinese study of 2007, phylogenetic analysis showed a close relationship of *C. magnoliae* to *Candida krusei*.

Conclusion: Immunosuppression with longstanding or repeated hospital admissions is a risk for nosocomial fungal infections, especially, bloodstream infections. Already confusing phenotypic identification among *C. glabrata*, *C. auris*, *C. haemoulonii*, and now the current isolate *C. magnoliae* further complicates and challenges diagnostic workflow impacting timely management of cases. Further studies and more documentation of such findings in literature are necessary for newer insights.

S6.2b

***In vitro* susceptibility testing of dermatophytes: toward standardization**

Ditte Marie Lindhardt Saunte[2]
[1]Department of Dermatology, Zealand University Hospital, Roskilde, Roskilde, Danmark
[2]Department of Clinical Medicine, Faculty of Health Science, University of Copenhagen, Copenhagen, Denmark

S6.2 Resurgence of dermatophytic infections, September 22, 2022, 4:45 PM - 6:15 PM

Antifungal treatment-resistant dermatophytosis has been known for years[1]. It has mainly been reported as sporadic cases with clinical failure to a specific antifungal confirmed by *in vitro* resistance to antifungal compounds determined by antifungal susceptibility testing (AFST). However, *in vivo* AFST of dermatophytes is not routinely available in most countries, and, therefore, many clinicians solve the problem by changing the antifungal treatment to another drug class hoping that it will result in clinical response. Unfortunately, cross-resistance revealing concomitantly reduced sensitivity to different classes of drugs including terbinafine and azoles have been reported[2,3]. Furthermore, an increase of antifungal resistant dermatophytosis has been noted mainly in India and other Asian countries[4], but sporadic cases have also been registered in the Middle East, Europe, and North and South America suggesting that this may be the top of the iceberg[5-8]. This stress the need for a standardized AFST, which can be used routinely in order to surveil the disease spread and implement targeted antifungal treatment.

Molecular-based methods are able to detect a genetic mutation known to cause antifungal resistance (e.g., mutation in the squalene epoxidase gene)[2] whereas culture-based AFST methods are able to determine the minimum inhibitory concentration (MIC) of a given drug for a specific clinical isolate. This should ideally enable to classify the isolate as sensitive, intermediate, or resistant to a specific antifungal agent, but unfortunately, it may be difficult to compare results across studies as the interpretations of MIC results are depending on the AFST method used. Following AFST methods have been used to determine the MIC of dermatophytes: E-test, agar dilution, agar disc diffusion, and macro- and microbroth dilution methods[9]. They differ in several ways including inoculum concentration, incubation temperature, incubation time, different culture media, and endpoint criterion of fungal growth (percentage of growth inhibition)[9,10]. Standardization is therefore important, and currently, two standardized guidelines for *in vitro* AFST of dermatophytes exist. One is from the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[11] and the other is from the Clinical Laboratory Standards Institute (CLSI)[12]. Both are using microtiter plates and the EUCAST (E.Def 11.0) guideline has included MIC breakpoints for the classification of whether an isolate is susceptible or resistant to a given drug. Unfortunately, breakpoints are only available for a limited number of dermatophytes and antifungals[11].

Standardized validated AFST methods will enable us to target the antifungal treatment thereby reducing the risk of ineffective and unnecessary exposure to inappropriate antifungals with potential side effects and reducing the risk of disease spreading.

Sources:

1. Michaelides P, Rosenthal SA, Sulzberger MB, Witten VH. *Trichophyton* tonsurans infection resistant to griseofulvin. A case demonstrating clinical and in vitro resistance. Arch Dermatol. 1961;83(6):988-90.
2. Martinez-Rossi NM, Peres NTA, Bitencourt TA, Martins MP, Rossi A, Mignon BR et al. State-of-the-Art Dermatophyte Infections: Epidemiology Aspects, Pathophysiology, and Resistance Mechanisms. 2021; Available from: https://doi.org/10.3390/jof7080629
3. Singh A, Masih A, Khurana A, Singh PK, Gupta M, Hagen F et al. High terbinafine resistance in *Trichophyton* interdigitale isolates in Delhi, India harbouring mutations in the squalene epoxidase gene. Mycoses. 2018 Jul;61(7):477-84.
4. Tang C, Kong X, Ahmed SA, Thakur R, Chowdhary A, Nenoff P et al. Taxonomy of the *Trichophyton* mentagrophytes/T. interdigitale Species Complex Harboring the Highly Virulent, Multiresistant Genotype T. indotineae. Mycopathologia. 2021 Jun 1;186(3):315-26.
5. Saunte D, Pereiro-Ferreirós M, Rodríguez-Cerdeira C, Sergeev A, Arabatzis M, et al. Emerging antifungal treatment failure of dermatophytosis in Europe: take care or it may become endemiC. J Eur Acad Dermatol Venereol. 2021 Jul 1;35(7):1582-6.
6. Süß A, Uhrlaß S, Ludes A, Verma SB, Monod M, Krüger C et al. [Extensive tinea corporis due to a terbinafine-resistant *Trichophyton* mentagrophytes isolate of the Indian genotype in a young infant from Bahrain in Germany]. Hautarzt. 2019 May 16
7. Lana AJD, Pippi B, Carvalho AR, Moraes RC, Kaiser S, Ortega GG et al. In Vitro additive effect on griseofulvin and terbinafine combinations against multidrug-resistant dermatophytes. Brazilian J Pharm Sci. 2018 Jan 1;54(2).
8. Chen E, Ghannoum M, Elewski BE. Treatment-resistant tinea corporis, a potential public health issue. Br J Dermatol. 2021 Jan 1;184(1):164-5.
9. Dogra S, Shaw D, Rudramurthy S. Antifungal Drug Susceptibility Testing of Dermatophytes: Laboratory Findings to Clinical Implications. Indian Dermatol Online J. 2019;10(3):225.
10. Curatolo R, Juricevic N, Leong C, Bosshard PP. Antifungal susceptibility testing of dermatophytes: Development and evaluation of an optimised broth microdilution method. Mycoses. 2021 Mar 1;64(3):282-91.
11. Arendrup MC, Kahlmeter G, Guinea J, Meletiadis J, Arendrup MC, Meletiadis J, et al. How to: perform antifungal susceptibility testing of microconidia-forming dermatophytes following the new reference EUCAST method E.Def 11.0, exemplified by *Trichophyton*. Clin Microbiol Infect. 2021 Jan 1;27(1):55–60.
12. Wayne P. CLSI. Reference Method for Broth Dilution Antifungal Susceptibility testing of Filamentous Fungi. 3rd Ed. CLSI standard M38 [Internet]. 2017. Available from: www.clsi.org.