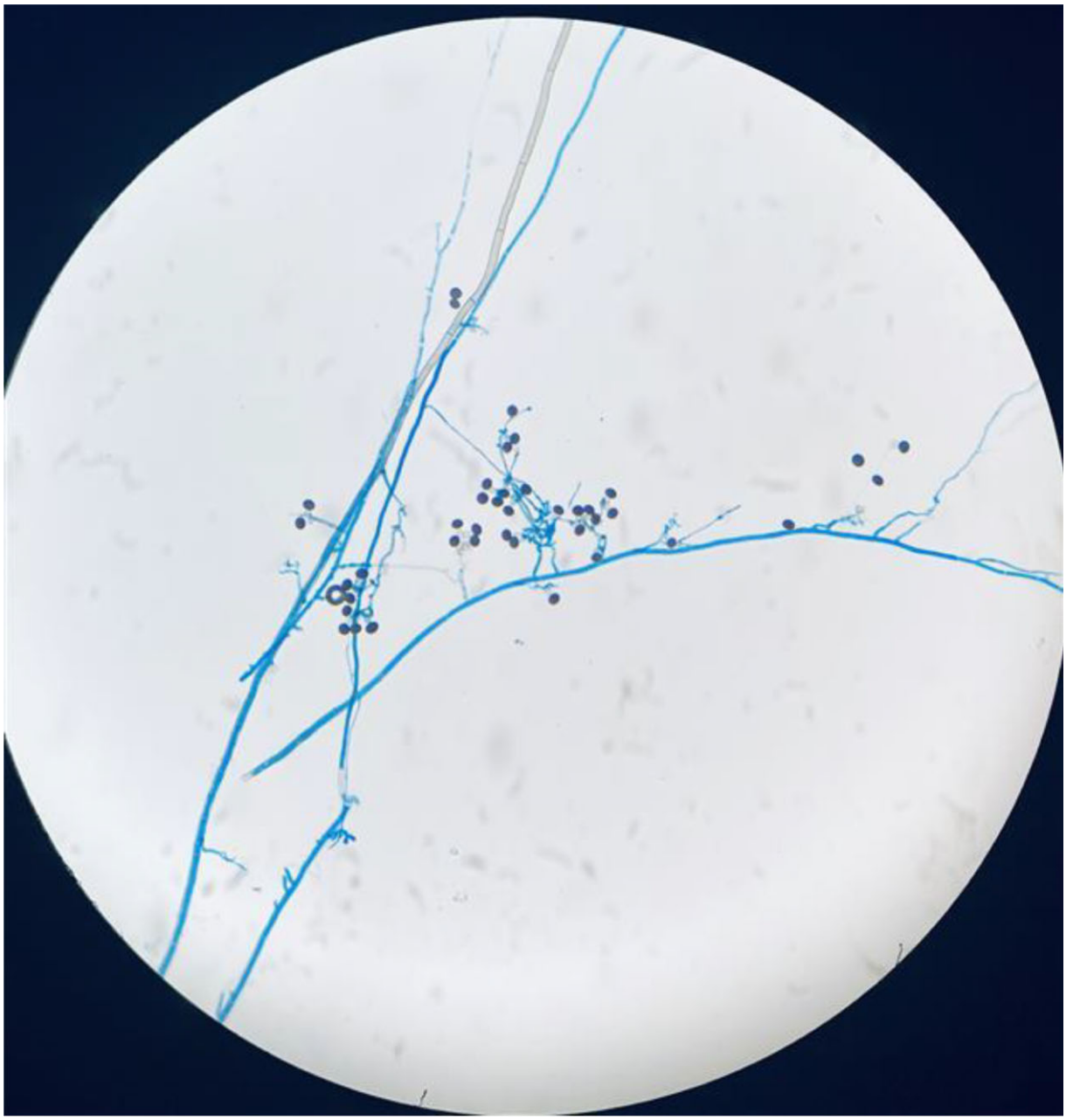

P257

Amplified fragment length polymorphism fingerprinting supports the absence of correlation of genotype with clinical phenotype or source of the isolate in *Aspergillus flavus* infections

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Poster session 2, September 22, 2022, 12:30 PM - 1:30 PM

Objectives: *Aspergillus flavus* accounts for ~ 10% of bronchopulmonary aspergillosis and is the second leading etiological agent of invasive aspergillosis worldwide. It is the commonest cause of fungal rhinosinusitis and ocular mycoses in tropical countries including India. We report amplified fragment length polymorphism genotyping of a set of clinical and environmental isolates to unravel the genetic diversity of *A. flavus* in India and to further determine correlation between isolate genotype and the source/clinical phenotype of the *Aspergillus* infection.

Methods: Two sets of morphologically identified isolates of *A. flavus* from clinical ($n = 71$) and environmental sources ($n = 22$) were included in the study using a stratified random sampling method. Clinical strains were isolated from lower respiratory tract specimens ($n = 22$), sinus and sino-nasal biopsies ($n = 25$), corneal samples ($n = 12$), and others ($n = 12$). Environmental strains were isolated from different niches like air, soil, and infected crop samples. *Aspergillus fumigatus* F145 was used as an out-group. DNA was extracted from the fungal broth culture following the method of Lee et al. AFLP was done as per an earlier described method using HpyCH41V and Mse1 (New England BioLabs) for restriction digestion followed by selective amplification of restriction-digested products with 1 mM HpyCH4 IV primer with one selective residue (5-Flu-GTA-GACTGCGTACCCGTC-3'), 1 mM MseI primer with four selective residues (5-GATGAGTCCTGACTAATGAA). Fingerprint data was analyzed in BioNumerics software version 7.6 (Applied Maths, Belgium). The phylogenetic tree was constructed using Pearson's Correlation coefficient. An AFLP genotype was assigned to a cluster using an arbitrary cut-off value of ≥90% fingerprint similarity. The distribution of genotypes among different categories was analyzed by Fisher's exact test or Pearson's χ 2 goodness of fit test with Yates correction factor as appropriate and two-tailed *P*-values of <.05 were considered statistically significant.

Results: The analyses revealed a total of 16 AFLP genotypes with 5 major clusters (≥ 5 isolates) reflecting the extent of genetic diversity in *A. flavus*. Genotype VIII encompassed predominantly clinical isolates (P <.01) and genotype XI with majority of isolates from environmental sources (P <.0001). The strains which were isolated from invasive and non-invasive forms or from different sites (pulmonary, sinus, and ocular) did not diverge into separate or unique clusters. Although the genotypes had an asymmetric distribution in different clinical presentations as revealed by the χ2 goodness of fit test, none of the genotypes was exclusively responsible for causing a particular infection. Isolates from the north zone of India shared genotypes with those from the southern region of the country. Three isolates formed a separate genotype XVI and diverged from the *A. flavus* cluster by 42% fingerprint similarity. Partial β-tubulin and calmodulin gene sequencing-based phylogeny reconstruction placed those three isolates in *A. tamarii* clade of the *A. flavus* species complex.

Conclusions: This study suggests that every genotype of *A. flavus* has the potential of causing an allergic, non-invasive or invasive infection. Further, *A. flavus sensu stricto* was predominantly (97%) isolated from clinical specimens revealing the majority of infections/colonization are caused by this species compared to other members in the Flavi complex.

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Incidence of Histoplasmosis, Cryptococcosis, and TB Among People Living with HIV in Paraguay-Preliminary Report

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Poster session 2, September 22, 2022, 12:30 PM - 1:30 PM

Objectives: Endemic fungal infections such as Histoplasmosis and Cryptococcosis as well as tuberculosis (TB) are important causes of mortality among people living with HIV (PLHIV) in Latin America. Rapid diagnostic assays (RDAs) could

Table 1. Opportunistic infections in patients with advanced HIV disease, Paraguay (2021-2022) ‡

Enrolled with <200 CD4 or WHO 3-4 stage	Histoplasmosis Ag positive	Cryptococcal Ag positive	TB-LAM positive*	Xpert positive**	TB confirmed by any method	Any Opportunistic infection	TB + Histoplasmosis co-infections	TB + Cryptococcosis co-infections	Histoplasmosis + Cryptococcosis co-infections
335	10 % (32/314)	11% (35/329)	20% 40(/196)	14% (15/108)	22% (51/232)	30% (100/335)	12/335 (3.6%)	3/335 (0.9%)	3/335 (0.9)

*Among those with <100CD4; **indicated for those with sputum production
‡ Based on the number of individuals with valid samples processed

decrease the time to diagnosis and treatment of these infections, resulting in a reduction in mortality. The objectives of this study were to determine the incidence of Histoplasmosis, Cryptococcosis, and TB using RDAs in PLHIV with advanced HIV disease (AHD) and calculate 30-day mortality.

Methods: PLHIV 18 years and older, treated at the Institute of Tropical Medicine hospital in Asuncion, Paraguay, not receiving ART and presenting CD4 count $\leq$ 200 cells/μL or clinical symptoms suggestive of WHO stage 3 or 4 diseases were enrolled and followed for 30 days. Detection of Histoplasma Ag (HisAg) in urine was performed by enzyme immunoassay (EIA), *Cryptococcus* Ag (CrAg) detection in serum and cerebrospinal fluid specimens by lateral flow assay (LFA), and lipara-binomannan (LAM) detection in urine by LFA (TB LAM) (limited to those patients with CD4 counts $\leq$ 100 cells/μL) and by GeneXpert (limited to patients with respiratory symptoms).

Results: From August 2021 to 25 March 2022, a total of 335 PLHIV were enrolled. Patient median age was 37 years [Interquartile Range (IQR) 16 years], median CD4 count at enrollment was 91 cells/μL (IQR 147 cell/μL). A total of 80% (n = 269) of patients were symptomatic for one or more of the three diseases being screened for. Ag positivity rate was 20% (40/196) for TB-LAM, 10% (32/314) for HisAg, and 11% (35/329) for CrAg (15 diagnosed with cryptococcal meningitis). GeneXpert testing showed a positivity of 14% (15/108), and six of these patients with positive GeneXpert also tested positive for TB-LAM.

In total, 100/335 (30%) of patients tested had a positive result and coinfections were observed among 14/335 (4.2%) patients (Table 1). Histoplasmosis + TB was the most frequent co-infection observed 12/335 (3.6%).

Mortality among those who completed 30-day follow-up was 12.6% (32/254) and 11% among those with an OI (11/102)

Conclusions: Preliminary results show that TB and fungal opportunistic infections, including co-infection were common in people with advanced HIV. Longitudinal follow-up will help to evaluate the feasibility and cost of implementing RDAs for the early detection of opportunistic infections in PLHIV with AHD in Paraguay. Early diagnosis could impact mortality reduction.

P259

Rare presentations of Cryptococcosis: a case series

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Objectives: *Cryptococcus* spp. is usually opportunistic pathogens affecting immunocompromised individuals causing meningitis primarily. Non-CNS presentations are a rare entity and we hereby present a series of 3 cases in the past 1 year (2021-2022).

Methods: Case records of the three patients were studied. Detailed history, demographic details, investigations, treatment were noted.

Results: Patient-1 was a 14-year-old girl who came with complaints of fever, pain, swelling, and restricted movements of the right wrist, elbow, and ankle joints with multiple subcutaneous swellings initially on the thigh followed by elbows, arms, and forearms. The swellings became hemorrhagic bullae bursting to form ulcers. She had a history of being treated 4 times for tuberculous lymphadenopathy. KOH-Calcofluor white mount of biopsy and pus aspirate samples showed circular yeast cells which were confirmed by cryptococcal antigen detection. All the samples had grown *Cryptococcus neoformans* on culture except blood, BAL, and CSF. She responded to Liposomal amphotericin-B drastically. Retesting of pus swabs from the ulcers after a week of antifungal therapy were negative for *C. neoformans*. Subcutaneous nodules and joint swellings decreased but she developed reactions to amphotericin B and was changed to fluconazole. She is on regular follow-up with no recurrence.

Patient-2 was a 22-year-old male, a known case of Hodgkin Lymphoma stage 4 who underwent Autologous stem cell transplantation (ASCT) and was on immunosuppressants. He presented with fever, dyspnea, and cough which got worsened along with multiple cervical, hilar and abdominal lymphadenopathy. KOH-Calcofluor white mount of biopsy samples demonstrated circular yeast cells which were confirmed by cryptococcal antigen detection test of biopsy and BAL samples. *Cryptococcus neoformans* was grown on culture from all the samples. He succumbed to ARDS and cardiorespiratory arrest before any treatment could be initiated.

Patient-3 was a 38-year-old female, known case of SLE with lupus nephritis, presented with intermittent fever, dyspnea, chest pain, decreased urinary output, and gradual swelling of the body starting from the face and progressing to the whole body. She further developed synpneumonic effusion, multiple erythematous tender papules over the right thigh, and cellulitis of the right lower limb. She was started on voriconazole in view of HRCT findings suggestive of fungal pneumonia. As galactomannan antigen test was negative, voriconazole was stopped. Pleural tap fluid flagged positive in Bactec and *C. neoformans* grown on subculture. Her condition worsened with septic shock and succumbed to the disease before any treatment could be initiated.

Conclusion: Subcutaneous, joint, and pulmonary involvement is rare, without a primary focus on the central nervous system. Culture and antigen detection can aid in early detection and hence early initiation of therapy.

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Sporotrichosis hyperendemic in Southern Brazil: twelve years of challenges

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Feline and zoonotic sporotrichosis has been described since the 1990s in the Rio Grande do Sul state (RS), southern Brazil. In reported cases, this region has the second-highest number of cases proven due to *Sporothrix brasiliensis* in the country.

Objective: We update the current situation of sporotrichosis in Southern Brazil and report measures taken to face the epidemiological threat of zoonotic sporotrichosis over 12 years.

Methods: Activities developed by the Laboratory of Mycology of the Universidade Federal do Rio Grande (LabMyco/FURG) and their results are described. Database from the LabMyco/FURG was consulted and all cases of proven sporotrichosis (required fungal isolation in culture) from humans and animals (cats and dogs) diagnosed between January 2010 and March 2022 were included.

Results: During the 12 years of the study, four educational events to discuss the regional emergence of sporotrichosis were promoted (in the years 2011, 2013, 2017, and 2018). Before these meetings, health professionals were interviewed, and approximately half were unfamiliar with the regional hyperendemicity, etiological agent, source of infection, and/or the main clinical presentation of sporotrichosis. With these events, a total of 144 health professionals were instructed to diagnose and treat the disease. Additionally, in 2017, along with the municipal health system, we implemented a public specialized reference service (SRS) at the University Hospital (UH) of FURG/Empresa brasileira de serviços hospitalares (EBSERH) to treat human sporotrichosis cases. The diagnosis of sporotrichosis was confirmed in 47 patients referred to UH-FURG/EBSERH. All were clinically evaluated by periodic follow-up until clinical cure and received free antifungal treatment by the Brazilian System of Health. A positive impact of the SRS was demonstrated by the decrease of 235% in the interval between the beginning of the lesions and diagnosis (before SRS, mean of 206 days versus after SRS implementation, 79.5 days). Since the start of sporotrichosis diagnosis by LAbMyco/FURG, January 2010-March 2022, 914 cases of proven sporotrichosis were diagnosed by fungal culture: 721 in cats, 135 in humans, and 58 in dogs.

Conclusion: In a region with >500 000 inhabitants, 914 cases of sporotrichosis were diagnosed in the last 12 years. Since all of these cases are from just a single laboratory service, we believe this number is underestimated, and the threat even greater. Although some improvements regarding the diagnosis and treatment of this mycosis in our region could be achieved by the educational activities and the implementation of SRS, the numbers are still alarming and there are several obstacles that urgently need attention. Our region has the second most important port of the country, thus, more efforts, with public health politics directed to the treatment of cats with sporotrichosis, are urgently needed to control the spread and fungal dissemination not just to local and regional populations, but also to other countries.

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***Candida* species in the bloodstream of patients from a tertiary hospital in southern Brazil**

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Candidemia in hospitalized patients, especially those admitted to intensive care units (ICUs), is responsible for prolonged periods of hospitalization and antifungal therapy, resulting in higher hospital costs and in high mortality rates. The knowledge of the local prevalence of *Candida* species in the bloodstream and its susceptibility profile is necessary for appropriate therapeutic and surveillance interventions.

Objectives: This study aims to evaluate the prevalence of candidemia in a tertiary hospital in southern Brazil over a period of around one and a half years, its etiology, and the susceptibility profile of the isolates to antifungal drugs.

Methods: A retrospective study was carried out at the University Hospital of Rio Grande (HU-FURG/EBSERH), which has 218 beds. All cases of candidemia, diagnosed by the isolation of yeasts in blood cultures (automated culture system—Bactec®) between January 2021 and April 2022, were included. Databases were analyzed to collect data regarding the total of blood cultures examined in the same period, as well as the etiology and its susceptibility profile to fluconazole (FLU) and amphotericin B desoxicolate (AMB) (microdilution assay according to M27-A3, CLSI).

Results: During the sixteen months of the study, 368 patients were examined by blood cultures in our hospital, being 216 from ICUs (n = 101 adult; n = 115 neonatal/pediatric). A total of 21 were diagnosed with candidemia, resulting in a prevalence rate of 5.7%. The majority of the candidemia cases (66.6% - 14/21) occurred in ICUs, including pediatric/neonatal ICU (6/115; 5.2%) and adult ICU (8/101; 7.9%). *C. albicans* was associated with 52.3% of the cases (n = 11). Among the non-*albicans* species (n = 10), four were identified through MALDI-TOF (*C. parapsilosis*: n = 3; *C. krusei*: n = 1). Antifungal susceptibility showed that 62.5% of the non-*albicans* isolates tested (6/8) were resistant to FLU or AMB.

Conclusions: *Candida* species are important pathogens associated with sepsis in our hospital, corresponding to around 5% of the bloodstream infections in patients hospitalized, independently of their unit of origin. These data raise awareness of the need for early diagnosis, surveillance of resistance and prevention of this bloodstream infection to optimize the treatment, and promote a better prognosis for critical patients.

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Clinico- microbiological profile of post-COVID pulmonary fungal infections encountered during the second wave of COVID-19 pandemic at a tertiary care teaching hospital in the Himalayas

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Objective: The study aims to generate preliminary data about post-COVID pulmonary fungal infections in the Himalayas and analyze patients' micro-radio-clinical profiles and outcomes.

Methodology: We conducted a retrospective study at a tertiary care teaching hospital in the Himalayas to generate preliminary post-COVID pulmonary fungal infection data. Sputum, Endotracheal Tube (ET), and Bronchoalveolar lavage (BAL) samples of patients sent to the Mycology laboratory were subjected to KOH mount and aerobic inoculation on Sabouraud dextrose agar plates at 37° C. The patients' symptoms, diagnosis, clinical-radiological profile, and outcome were collected from the hospital database.

Results: Among n = 16 cases of post-COVID pulmonary fungal infections aged 53 +/- 13.38 years, n = 7 (43.75%) had Pulmonary Aspergillosis (n = 5 *A. fumigatus*, n = 1 *A. flavus*, n = 1 *A. niger*), n = 5 (31.25%) had Pulmonary Mucormycosis (*Rhizopus arrhizus*), and n = 4 (25%) had mixed infection. In 2 of 4 mixed infection patients, *R. arrhizus* was identified on KOH microscopy and *A. fumigatus* on SDA Agar. Both *A. fumigatus* and *R. arrhizus* were identified on KOH Microscopy of