Objectives: Aspergillus flavus accounts for ~10% of broncho-pulmonary aspergillosis and is the second leading etiological agent of invasive aspergillosis worldwide. It is the commonest cause of fungal rhinosinusitis and allergic mycoses in tropical countries including India. We report amplified fragment length polymorphism genotype 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 associated clinical phenotype of the Aspergillus infection.

Methods: Two sets of morphologically identical 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), cerebral samples (n = 12), and others (n = 12). Environmental strains were isolated from different niches like air, soil, and insect crop samples. Aspergillus flavus genome F45 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 HpyCH4IV and MseI (New England Biolabs) for restriction digestion followed by selective amplification of restriction-digested products with 1 μm HpyCH4 IV primer with one selective residue (5′-GATGACTGCTCTCTGTACAATGAA). 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 ≥95% fingerprint similarity. The distribution of genotypes among different categories was analyzed by Fisher’s exact test or Pearson’s χ² 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 (≥ 1 isolate) reflecting the extent of genetic diversity in A. flavus. Genotype VII encompassed predominantly clinical isolates (P < .01) and genotype XI with majority of isolates from environmental sources (P < .0001). The strains which were isolated from naso and sino-nasal forms or from different sites (pulmonary, sinus, and cerebral) 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 were exclusively responsible for causing a particular infection. Isolates from the northern state of India shared genotypes with those from the southern region of the country. These 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 Flavus complex.

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

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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 (RDA) could
discuss 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 RDMS in PLWH with advanced HIV disease (AHD) and calculate 30-day mortality.

Methods: PLWH 18 years and older, treated at the Institute of Tropical Medicine Hospital in Asuncion, Paraguay, not receiving ART and presenting CD4 count < 200 cells/μl, or clinical symptoms suggestive of WSSH stage 3 or 4 diseases were enrolled and followed for 30 days. Detection of Histoplasma Ag (Histag) in urine was performed by enzyme immunoassay (EIA). Cryptococcus Ag (ELISA) detection in sputum and cerebrospinal fluid by lateral flow assay (LFA) and IgG and IgM antibodies in serum by LFA (TB-LAM) limited to patients with CD4 count ≤ 100 cells/μl and by GenXpert (limited to patients with respiratory symptoms).

Results: From August 2020 to 23 March 2022, a total of 335 PLWH were enrolled. Median age was 47 years (interquartile Range [IQR] = 14 years, median CD4 count at enrollment was 91 cells/μl) (IQR = 147 cells/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% (60/300) for Histag in urine, 15% (45/300) for TB-LAM, 10% (30/300) for Histag in urine and 11% (33/292) in CD4 (5 diagnosed with cryptococcal meningitis). GenXpert testing showed a positivity of 14% (17/121) and was in 36% of the patients with positive GenXpert also tested positive for Histag in urine.

In total, 100/335 (30%) of patients tested had a positive result and confounding factors were observed among 14/131 (11%) patients (Table 1). Histoplasmosis: 8 TB was the most frequent co-infection observed 12/335 (3.6%). Multiple mycoses among these patients: 42/335 (12.6%) in histoplasmosis, 14/335 (4.2%) in cryptococcosis and 3/335 (0.9%) in tuberculosis. 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 RDMS for the early detection of opportunistic infections in PLWH with AHD in Paraguay. Early diagnosis could impact mortality reduction.

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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 rarity 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 22-year-old girl who came with complaints of fever, pain, swelling, and restriction movements of the right wrist, elbow, and ankle joints with multiple subcutaneous swellings initially on the thigh followed by elbows, arms, and torso. The swellings became homogenous, indurated, and fixed to bone. She had a history of being treated 4 times for tuberculosis lymphadenopathy. KOH-Crystal violet of the specimen showed characteristic budding yeast cells which were confirmed by cryptococcal antigen detection. All the samples had grown Cryptococcus neoformans on culture except BSM and CFS. She responded to itraconazole and amphotericin B dramatically. Repeating of pus ooze from the cultures after a week of antifungal therapy was negative for C. neoformans. Swellings in subcutaneous and joint swellings decreased but she developed reactivation symptoms in BSM-P and PBD was changed to itraconazole. She is on regular follow-up with no recurrence.

Patient 2 was a 22-year-old male, a known case of HIV from a local hospital stage 4 who underwent Antiretroviral therapy (ART) at 19 years of age for the last 5 years. He presented with swelling in multiple sites on the arm and the leg due to lymphadenopathy. KOH-Crystal violet of the specimen showed budding yeast cells which were confirmed by cryptococcal antigen detection test of biopsy and BAL samples. Cryptococcus neoformans grew 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, short spells, decreased auditory output, and gradual swelling of the body starting from the face and progressing to the whole body. She further developed symptomatic effusion, multiple erythematous tender papules over the right thigh, and cellulitis of the right lower limb. She was sent on rosuvastatin in view of HBCS findings suggestive of fungal pneumonia. As galactomannan test was negative, vasoactive drugs were started. Pleural tap had flagged positive in BAC and C. neoformans grown on subculture. Her condition worsened with septic shock and succumbed to the disease before any treatment could be initiated.

Conclusions: Subcutaneous, joint, and pulmonary involvement in 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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Sporotrichosis has been described since the 1930s in the Rio Grande do Sul state (RS), southern Brazil. In reported cases, this region has the second-highest number of cases due to S. brasiliensis in the country.

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

Methods: Authors developed by the Laboratory of Mycology of the Universidade Federal do Rio Grande (LabMyco/FURG) and their results are described. Data from the LabMyco/FURG were combined 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 2013, 2015, 2017, and 2018. Before these meetings, health professionals were intervened, and approximately half were unaware with the regional hyperendemic zoonotic agent, source of infection, and/or the usual 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 (ABT – Ambulatório de Referência em Tratamento de Serviços Hospitalares – EBSERH) to treat human sporotrichosis cases. The diagnosis of sporotrichosis was confirmed in 47 patients referred to UFRG/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 EBS was demonstrated by the decrease of 23.5% in the interval between the beginning of the lesions and diagnosis (before: 306 days versus after EBS implementation, 79.5 days). Since the start of sporotrichosis diagnosis by LAB-Mycologia, January 2010-March 2022, 114 cases of proven sporotrichosis were diagnosed by fungal culture: 721 cases in cats, 135 in humans, and 58 in dogs.

Discussion: In a region with 600,000 inhabitants, 22% of 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 events and health promotion activities, the diagnosis and treatment of sporotrichosis in this region remains a scourge that urgently needs attention. Our region has the second most important part of the country, thus, more efforts, with public health policies directed to the treatment of cats with sporotrichosis, are urgently needed to control the spread and fungal dissemination not just to local and regional population, but also to other countries.