Molecular epidemiology of environmental strains of Cryptococcus isolated from Varanasi, Uttar Pradesh, India
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Poster session 2, September 22, 2022, 12:30 PM - 1:30 PM

Objectives: Cryptococcus affects more than one million people per year worldwide. Despite the worldwide emergence of this ubiquitous fungus, little is known about the global molecular epidemiology of this fungal pathogen. The ubiquitous nature of the ovoidal agglutinins of C. neoformans and C. gattii are not well established yet in various parts of India.

Methodology: The present study was performed on the C. neoformans isolated from the Eucalyptus tereticornis at Varanasi, India. A total of 245 samples including leaves, bark, and nearby soil of E. tereticornis. The fungal pathogens were identified by both the conventional and molecular methods. The isolates were also grown in Brain’s Y and tobacco agar medium. In addition, all isolates were identified on the basis of different biochemical tests such as urease test and cyanoamino-glycine-bromothymol blue (CGB). Further, molecular characterization was carried out by using PCR and DNA sequence analysis.

Results:
- 80% (195) of the isolates were positive for C. neoformans.
- 77.5% (190) of the isolates grew on the agglutinin medium.
- 74% (180) of the isolates were positive for C. neoformans.
- The presence of brown colonies on Brain’s Y and tobacco agar media confirmed that the isolated pathogen is Cryptococcus.
- Further, the isolates of Cryptococcus were tested for capsule production using Chitinase assay on agar medium in which all isolates were found to hydrolyze agar rapidly and reddish pink color was obtained confirming it to be C. neoformans.
- All isolates were negative for the presence of C. neoformans var. grubii.
- Molecular typing grouped the isolates into two major genotypes. One was molecular type VNI (serotype A, var. grubii), one of the isolates was molecular type VNII (serotype D, var. neoformans).
- Figure 3 shows the gel electrophoresis images of ITS1-5.8S amplicons.
- The nucleotide sequences were submitted to NCBI GenBank with accession numbers: MZ824412, MZ841845, MZ825511, MZ825179, MZ825572, and MZ831796.

Conclusion: In spite of a major Eucalyptus growing region of India, there is no report of C. neoformans/C. gattii-Eucalyptus association from Uttar Pradesh. Therefore, the current study would certainly be helpful in the establishment of molecular epidemiology of Cryptococcus in this area.

African Woman With a Knee Knobbed by a Rare Dematiaceous Fungi
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Poster session 2, September 22, 2022, 12:30 PM - 1:30 PM

Objective/Introduction: Incidence of fungal post-prosthetic joint infection (PJI) is rare (<1–5%) and the majority are caused by Candida and Aspergillus. We report a post-prosthetic knee joint infection caused by a rare dematiaceous fungus—Phaeoacremonium richardiae, probably the first case in the world.

Methods/Case Details: A 79-year-old East African female from Malawi, however, with no known medical comorbidities presented with a chronic history of left knee pain and pus discharge. She had left knee pain since 2008 and was given several intra-articular injections between 2008 and 2010 for pain relief suspecting osteoarthritis. She denied a history of splinter injuries, trauma, systemic, or constitutional symptoms.

In 2010 she underwent left total knee replacement (TKR) in the USA, but the pain persisted post-operatively associated with amniostomy swelling of both knees. She was evaluated again in 2015 and revision left TKR done with single-stage exchange. She was asymptomatic for a few years but symptoms returned again and drainage of pus from her left knee started in December 2019. She was treated in Malawi with multiple courses of parenteral and oral antibiotics but did not improve. She presented to our hospital in January 2022 with swelling in left knee and restriction of movements. On examination, a draining sinus was noted over the medial aspect of left knee. She was afebrile with a normal leucocyte count, Hb, negative, ESR of 81 mm, and CRP of 23 mg. Her renal and liver function tests were normal. CT left leg with intravenous showed features of chronic osteomyelitis of left tibial funiculum and proximal tibia with active sinus tract in left tibia.

Surgical excision with removal of prosthesis, debridement, and antibiotic cement spacer insertion was done. Bone and post-prosthetic tissue were sent for histopathology and microbiological analysis including fungal and mycobacterial cultures. Xpert MTB was negative.

Histopathology showed granulomatous reaction with fungal hyphae and spores. Cultures grew a slender septate dark pigmented fungus, Phaeoacremonium richardiae which was confirmed by fungal PCR sequencing of internal transcribed spacer (ITS) region.

Results/Treatment: She was treated with Liposomal amphotericin B 5 mg/kg IV ID for 2 weeks followed by oral Itraconazole. She had persistent raised inflammatory markers at 4 weeks which settled after changing to posaconazole for 2 weeks. Conservative management will continue for 3-4 months with second stage revision arthroplasty/arthrodesis later.

Conclusion: Dimatiaceous fungi usually cause skin and soft tissue infections and they are extremely rare in causing prosthetic joint infection. Case reports of P. richardiae causing osteomyelitis of foot and midfacial bones are available but we couldn’t find a published case of prosthetic joint infection caused by it. Identifying the causative organism in PJI is the most important step because the management depends mainly on it.

Two-stage exchange in combination with antifungal administration between stages and post-revision should be the presurgery choice for fungal PJI. Incorporation of antimicrobial agents into cement spacers appears to be effective in eradicating local infections and reducing the duration of antifungal treatment and should be strongly considered.
Comparison of clinical presentation of 2 cases of paracoccidioidomycosis in Shushufindi, Ecuador

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

Objectives: Discuss the diagnosis of two cases of paracoccidioidomycosis (PMC) for the first time in the city of Shushufindi, Ecuador.

Report two different clinical presentations in two male patients diagnosed with PMC.

Methods: The diagnosis and treatment of two male patients with PMC is retrospectively described. Individual informed consents were obtained from each patient.

Results: The first case is a 39-year-old male patient, with a history of previous armadillo hunting, with no relevant history. Born and resident in Shushufindi, Amazon area of the province of Sucumbíos, Ecuador. He presents lesions of ~7 years of evolution, characterized by ulcerated plaques with necrotic crusts in the nose and in the mouth at the level of the upper lip (Figs. 1a and b).

This patient had had several visits to different medical doctors, who unfortunately diagnosed other diseases. Due to his long clinical evolution, he was referred to a dermatological referral center in Quito. During the initial examination, a differential diagnosis of oncologic pathology, mycosis, mycobacteria, and cutaneous leishmaniasis was considered. A punch biopsy of the affected region was requested, and specimens were sent for culture, histopathology, and smears from the margins of the lesion to rule out leishmaniasis. As shown in Figure 2a, a histopathology report was submitted with yeast-like structures with multiple branches and a positive culture for Paracoccidioides (Fig. 1d). Other clinical laboratory studies or chest imaging were within normal parameters.

The second case is a 54-year-old male patient, a farmer, with no relevant history. Born and resident in Shushufindi. No clinical or geographical relationship with case number 1. He presented lesions of ~2.5 years of evolution, characterized by ulcerated plaques with necrotic crusts in the lower part of the nose, with lytic destruction of the anterior nasal septum and left nasal ala (Figs. 2a and b).

This patient has also consulted many physicians. At his first visit, he was treated for cutaneous leishmaniasis without a positive result. He was admitted to a specialized dermatological center in the city of Quito. 3 weeks after the first patient, a similar protocol was used. Patient number two had a slight elevation of liver enzymes, other laboratory, and imaging tests were within normal parameters. Unfortunately, cultures showed no fungal growing, and histopathology of a punch biopsy of the nasal septal mucosa showed yeast-like structures with multiple extensions suggestive of PCM (Fig. 2c).

In both patients, treatment was started with oral trimethoprim-sulfamethoxazole 200 mg per day. In the first 2 months, the treatment showed little success, so it was decided to increase trimethoprim-sulfamethoxazole to 8 mg/kg per day for 6 months.

Conclusion: Paracoccidioidomycosis is an endemic disease in South America characterized by infection with Paracoccidioides brasiliensis. Several articles have described cases in Ecuador, but this is the first report from the city of Shushufindi. The patients had different orofacial manifestations. In the patient with the shortest duration of infection, lytic damage to the nasal septum was observed (Fig. 2b).