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COVID-19-a associated mucormycosis: an experience of invasive fungal sinusitis from a tertiary care referral center in Northern India

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Objectives: The study was aimed to determine the patient demographics, risk factors, which include comorbidities, presenting symptoms and signs, medications used to treat COVID-19, species of Mucorales isolated, and the management outcome of COVID-19-associated mucormycosis.

Methods: It was a 6-month retrospective, propensity score-matched, comparative study conducted at a tertiary care center, involving 124 patients with COVID-19 associated mucormycosis admitted between April and September 2021, who were suffering from or had a history of COVID-19 infection.

Results: Among the 124 patients, 87 were male, and 37 were female. A total of 72.6% of patients received steroids, while 73.4% received antibiotics, and 55.6% received oxygen during COVID-19 management. The most common comorbidities were diabetes mellitus (83.9%) and hypertension (30.6%). A total of 92.2% had mucor, 16.9% had *Aspergillus*, 12.9% had both, and one patient had hyalohyphomycosis on fungal smear and culture. The comparative study showed the significant role of serum ferritin, glycemic control, steroid use, and duration in COVID-19-associated invasive fungal disease ($P < .001$). Headache and facial pain (68, 54.8%) were the most common symptoms. The most involved sinonasal site was the maxillary sinus (90, 72.6%). Direct KOH microscopy for fungus was positive in 100% of the cases, 82.2% were Mucorales; 12.9% showed mixed fungal hyphae (mucormycetes and *Aspergillus* spp.), 4% *Aspergillus* and hyaline septate non-*Aspergillus* fungus hyphae were seen in 0.8%. Mucoraceous fungi isolated were *R. arbuscus*, *R. microsporus*, *R. homothallicus*, *Rhizomucor pusillus*, and *Leitheimia corymbifera*. The overall survival rate at the 3-month follow-up was 79.9%.

Conclusion: COVID-19-related aggressive inflammatory response, uncontrolled glycemic level, and rampant use of steroids are the most important predisposing factors in developing COVID-19-associated invasive fungal sinusitis caused by Mucorales. A high index of suspicion, aggressive surgical and antifungal therapy resulted in the successful outcomes for most of the patients.

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Rhino sinusoidal Lasiodiplodia theobromae: a clinical and diagnostic dilemma

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Objectives: To disseminate and discuss a rare case of rhino sinusoidal *Lasiodiplodia theobromae* in a known diabetic.

Method: A case study was prospectively done since 2021 following a hospital visit of a 56-year-old male patient presenting with rhino sinusitis mimicking mucormycosis. Ethical approval and patient consent were obtained for this study. A detailed account of case progression from the date of first hospital admission to the final cure was noted. A 56-year-old male from Madhya Pradesh, in central India, presented to the outpatient department of Otorhinolaryngology (ENT) -Head and Neck Surgery. The patient was a known case of diabetes for 5 years. He presented with a headache for 10 days, throbbing pain over the left cheek and upper teeth with nasal discharge and crusting for one week. Nasal crusting and sample from left maxillary meatus were sent suspecting rhino sinusoidal mucormycosis. Mycological evaluation by KOH wet mount showed melanized hyaline and short fragments appearing broad pauciseptate. After 10 days, a grey fluffy growth was observed at 37°C and 25°C on SDA with chloramphenicol. On SDA with cycloheximide there was no growth. LPCB mount of the growth showed some hyaline hyphae with immature hyaline conidia and at places brownish dark walled single septate conidia. Phenotypic identification was inconclusive. Debridement surgery was done and amphotericin B was initiated. The patient was discharged after 10 days with the mention of routine follow-up. The isolate was sent to PGIMER Chandigarh Mycology Reference Center India for species confirmation. After almost 3 months the species was confirmed as *L. theobromae*.

Results: After almost 1-year clinical improvement was observed.

Conclusion: This study addresses the diagnostic dilemma both for the clinicians as well as clinical mycologists. Also, it emphasizes the need for molecular diagnostic workflow in such a scenario.

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Molecular epidemiology of Cryptococcosis—over 14 years using Whole Genome Sequencing and Multi-Locus Sequence Typing

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Objectives: *Cryptococcus neoformans*/*Cryptococcus gattii* causes Cryptococcosis—a systemic fungal disease in immunocompromised as well as immunocompetent individuals.

Whole genome sequencing (WGS) technology provides insights into the molecular epidemiology of *Cryptococcus* species. The main objective of this study was to analyze the genetic variations between the *Cryptococcus* species isolated in our center from patients from different parts of India using WGS and Multi-Locus Sequence Typing (MLST).

Methods: This was a study done on 56 strains of *Cryptococcus* that had been previously identified as *C. neoformans* from patients who were treated at Christian Medical College from 2007-2020. The organisms were identified by conventional colony morphology and microscopy and species identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). To determine the genetic diversity of *C. neoformans* isolates and the genetic structures of their populations, these *Cryptococcus* species isolates were further subjected to whole genome sequencing (WGS) using the Illumina platform followed by MLST using seven different housekeeping genes for genotyping the isolates. Based on the analysis, the *Cryptococcus* species isolates were characterized into Molecular types and clonal types, and their prevalence calculated.

Results: In this study out of the 56 *Cryptococcus* isolates, 50 were *C. neoformans* and 6 were *C. gattii*. A total of 46% of the patients from whom the *Cryptococcus* species were isolated from Eastern states of India and 42% were from South India. Out of the 50 *C. neoformans* 24 (48%) were isolated from patients infected with Human immunodeficiency virus (HIV), (14%) from those with autoimmune disorders, 6 (12%) from patients with post renal transplant on immunosuppressants. A total of 5/6 *C. gattii* patients were immunocompetent. The most common clinical presentation was meningoencephalitis with the CSF being the most common clinical sample from which the organism was isolated. The phylogenetic tree was constructed to identify the genetic similarities between the isolates. A total of 49/50 *C. neoformans* belonged to molecular type VNI (98%) and one was type VII (2%). They were also sub-typed into 11 sequence types including one novel sequence type (ST). A total of 44% of them belonged to the sequence type (ST) 93 followed by ST 5 (14%). WGS analysis of *C. gattii* is ongoing.

Conclusion: Molecular epidemiology of *Cryptococcus* species WGS and MLST is an important tool to understand the genetic diversity of *Cryptococcus* isolates in India over the years. Further studies on identifying the virulence factors and drug resistance mutations are needed to further understand the molecular mechanisms of disease pathogenesis.

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Molecular identification and antifungal susceptibility pattern of Candida species isolated from pediatrics with high colonization index in the Children Medical Center, Tehran

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Objectives: Candidemia is the third leading cause of bloodstream infections whereas it is the second leading cause of sepsis-related mortality in children and subsequently, understanding a link between such infection and *Candida* colonization is an important issue for clinicians for decades; so, this link has proposed the colonization index as the 'missing link'. The aim of this study was to evaluate the *Candida* colonization index in pediatric patients staying at intensive care units (ICUs) as well as bone marrow transplantation (BMT) unit and to determine the species distribution and susceptibility pattern of the isolates against several antifungal drugs.

Material and Methods: This study was conducted in the Children's Medical Center in Tehran-Iran between March 2019 and September 2019. Totally, 661 samples from 83 patients including oral cavity, skin surrounded catheters; ear, throat, nasal, and urine cultures were collected. *Candida* colonization index (CI) was calculated according to the previous study's description. CHROMagar *Candida* medium was applied for primary/multiple species identification and the isolates were then identified by using PCR-based methods to the species-specific complex level. Antifungal Susceptibility test was performed according to the Clinical and Laboratory Standards protocol published as M27-A2 and M60 documents. Fluconazole (FLZ), itraconazole (ITZ), voriconazole (VRZ), ravuconazole (RVZ), and isavuconazole (ISZ), caspofungin (CAS) and anidulafungin (AFG), and amphotericin B were used.

Results: Of 661 samples, 178 samples from 50 individuals (27%) were considered as positive cultures, amongst CI >0.5 confirmed in 29 cases (58% of positive samples). *Candida albicans* was reported as the most frequent species in BMT and ICU. Notably, *C. krusei* was the most prevalent species in PICU and the second in ICU. *Candida albicans* ($n = 53$, 49.5%) followed by *C. glabrata* ($n = 20$, 18.7%), *C. krusei* ($n = 15$, 14%), *C. parapsilosis* ($n = 12$, 11.2%) were the most frequent *Candida* species in patients with CI >0.5. Among FLZ-resistant strains, 12 isolates (7.01%) were multi-azole resistant which showed high MICs against both ISZ and RVZ and 7 strains (4.09%) were resistant to all echinocandins. ISZ, RVZ, and AFG were reported as the most effective antifungals, regarding the values of GM.

Conclusion: In the present study, more than half of children with positive yeast cultures had CI >0.5 representing the high possibility of developing invasive candidiasis. However, none of the children with CI ≥ 0.5 developed invasive candidiasis during the time of the study. To the best of our knowledge, there is no study about the evaluation of CI in pediatrics in Iran. More detailed and larger studies should be made to understand the impact of risk factors on CI value in the future.

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Candida nivariensis infection in South East Asia: series of case reports from Selayang Hospital, Malaysia and South East Asia Region

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Introduction: *Candida nivariensis*, first described from Spain in 2005 is an emerging fungal pathogen. Malaysia has captured a total of 11 cases since its first reported here in 2014. Indonesia, Thailand, and Vietnam are among South East Asia countries that reported its occurrence too.

Objectives: The occurrence and characteristics of *Candida nivariensis* in South East Asia are studied. We would like to highlight two cases from Selayang Hospital, Malaysia that occurred during pandemic COVID-19 in 2020 and 2022.

Case Description: In July 2020, a 60-year-old lady undergoing poorly controlled diabetes mellitus, CKD stage V approaching ESRD, congestive cardiac failure, hypertension, and dyslipidemia was diagnosed as complicated MSSA bacteremia secondary to right gluteal carbuncle. Appropriate antibiotics were commenced. Aggressive source control including incision and drainage, cauterization of right gluteal carbuncle, and multiple wound debridement for the infected gluteal wound was performed. She underwent a trephine sigmoid loop colostomy. Tracheostomy was done following prolonged ventilation. Anidulafungin as empirical fungal coverage was commenced in ICU as she further deteriorated. *Candida nivariensis* was isolated later from her blood culture.

In February 2022, a 74-year-old man underlying Diabetes mellitus, chronic kidney disease, hypertension, ischemic heart disease with two vessels disease stented, post-Whipple's procedure due to necrotizing pancreatitis in 2014 complicated with chronic abdominal pain with hyperalgesia admitted to the ward for acute right lacunar infarct with failed swallowing test and neurogenic bladder. His general condition deteriorated with worsening of biochemical and septic parameters. His blood culture grew *C. nivariensis* following which anidulafungin was started. No distant seeding and no signs of endophthalmitis. He then required mechanical ventilatory support complicated with multiorgan failure, leading to ICU admission.

Methods: For both cases, yeast isolates were subcultured on Sabouraud dextrose agar and CHROMagar. Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) with molecular testing was performed for confirmation of isolates. Phylogenetic tree among isolates from Malaysia was analyzed. Antifungal susceptibility test (AFST) to study the minimum inhibitory concentration (MIC) among treatment options was performed using the e-test method.

Results: Wide use of MALDI-TOF MS with molecular testing increased the detection rate of the species. Isolation sites in Malaysia and South East Asia region include blood culture, peritoneal fluid, high vaginal swab, and oropharyngeal swab. Risk factors namely immunocompromised status, indwelling vascular catheter, abdominal surgeries, renal failure or on dialysis support, polymicrobial usage, and prolonged stay in ICU. AFST established susceptibility towards fluconazole, high MIC to itraconazole and low MIC against amphotericin B, anidulafungin and voriconazole for both patients who unfortunately succumbed to their illness.

Conclusion: Diagnosis of *C. nivariensis* is challenging due to its closely related phylogenetic with *C. glabrata*, and *C. braccarensis*, thus molecular testing is vital. Inadequate antifungal coverage or delay of treatment could be detrimental to patient's outcome. Continuous epidemiological surveillance is crucial to address this potential invasive fungal pathogen and to observe emerging of drug resistance.

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Autochthonous cases of Madura foot from Jodhpur, Western Rajasthan, India

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Mycetoma has been classified as a neglected tropical disease by the WHO. 'Madura foot' is a prominent presentation of phaeoid fungi *Madurella* complex in India. Madurellomycetoma is frequently encountered in Western Rajasthan, where it is endemic. A high prevalence in this area is due to the desert climate of prolonged hot sunshine and scanty annual rainfall. The classic clinical 'triad of Mycetoma' involves a painless hard swelling, multiple fistulas, and discharge of grains.

We report two cases of eumycetoma from Western Rajasthan:

Case Report 1: A 41-year-old male, farmer presented a history of progressive swelling of the left foot in the last 4 years following trauma to the left sole 5 years ago. Patient took multiple treatments with no improvement. General examination showed a pallor and inguinal lymphadenopathy. Local examination revealed tumefaction with multiple sinuses discharging serousanguinous fluid with occasional black granules. Laboratory tests revealed hemoglobin of 6.2 g/dL. Serology was positive