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Investigation of an outbreak of neonatal *Candida* emia in the NICU of a 300 -bedded hospital in North India

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Objectives: Neonatal *Candida*emia causes significant morbidity and mortality in very low birth weight neonates. We report the occurrence of an outbreak of neonatal *Candida*emia due to *Candida krusei* in the neonatal intensive care unit (NICU) of a 300-bedded hospital in North India.

Methods: A total of 96 blood cultures from 80 neonates admitted in the NICU from October 2020 to April 2021 were received and processed manually in the Microbiology lab. A total of 5 among the 47 yeast isolates were sent to a teaching hospital for identification and antifungal susceptibility testing by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). Clinical parameters (very low birth weight, gestational age, birth asphyxia, broad-spectrum antimicrobial therapy, intra-uterine growth restriction, and total parenteral nutrition), lab parameters (CRP, platelet count), and patient outcome were evaluated. A total of 26 environmental samples and hand swabs from six health care workers were collected to trace the source of the outbreak. The samples were inoculated on 5% sheep blood agar and MacConkey agar. Regular rounds of the NICU and training sessions were conducted by the infection control team to intensify the infection control measures.

Results: Blood culture results: In all, 57 neonates (71.25%; 57/80) admitted to the NICU had positive blood cultures. A total of 47 blood cultures yielded non-*albicans Candida* spp. (82.45%; 47/57). The other microorganisms isolated from blood cultures were Pseudomonas aeruginosa (4), MRSA (3), Klebsiella pneumoniae (4) and Acinetobacter baumanni (1). Polymicrobial bloodstream infections were detected in two neonates. All the yeast isolates subjected to MALDI-TOF were identified as *Candida krusei* and had similar MICs of 0.25, 4, 16, 1, 0.25 and 16 μg/ml for voriconazole, itraconazole, posaconazole, caspofungin, micafungin and fluconazole respectively.

Culture results of environmental sampling: Yeast could not be isolated from any environmental sample and hand swabs of health care workers. However, MRSA was isolated from the hands of one health care worker.

Observation during the infection control rounds:

1. Sterile gauze pieces soaked in iodine and kept in plastic bottles were being used for skin antisepsis before collecting samples or before introducing an IV cannula.
2. The nursing staff did not wear sterile gloves while collecting blood samples or introducing IV cannula in case of NICU patients.
3. It was observed that the used bottles of IV fluids (eg. Ringer lactate, normal saline) were not capped and kept at room temperature.
4. Intensive infection control measures and training sessions were effective in controlling the outbreak.

Conclusion: We report the occurrence of a massive outbreak of neonatal *Candida*emia in the NICU of our hospital with a positivity rate of 58.75% (47/80). Identification of yeast and determination of antifungal susceptibility is important for prompt treatment. Though the source of the outbreak could not be traced but intensifying infection control practices could control the outbreak. This study emphasizes the value of educating healthcare workers and regular monitoring of disinfection practices to prevent health-care-associated infections.

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Rare isolates from subcutaneous mycotic lesions; A study from tertiary care center in Chhattisgarh, India

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Aims and Objective: To identify the causative agents of suspected subcutaneous mycosis patients attending to a tertiary care hospital, Chhattisgarh, India.

Introduction: Subcutaneous mycoses are a group of fungal infections of dermis and subcutaneous tissue caused by both melanized and hyaline molds. It often affects patients with immunosuppressive conditions. It consists of *Sporotrichosis, Chromoblastomycosis, Phaeohyphomycosis, Hyalohyphomycosis, Mycetoma,* subcutaneous *zygomycosis, Rhinosporidiosis, Lobomycosis,* and disseminated *Penicilliosis*. There are proven pathogenic agents causing subcutaneous mycosis though are not regularly isolated and reported. Few of them are commonly come across in the laboratory. Herewith, emphasized on the unusual clinical isolates from the patients having subcutaneous mycotic lesions with their clinical details.

Method: It is a retrospective descriptive analysis of data of subcutaneous mycosis cases of duration January 2019 to March 2022.

Total 52 clinical specimens from the suspected subcutaneous mycotic lesion were studied. Male dominance was observed amongst the patients. Amongst 52, 31% were detected positive for fungal elements by direct microscopy in 20% KOH mount, 55.7%, 25% positivity was observed in Culture and by both KOH wet mount and culture. Samples were processed and identified by using standard protocol.

The significant unusual isolates identified were *Conidiobolus coronatus* from subcutaneous cyst from buttocks, *Medicopsis romerai* from fine needle aspiration from the nodule of left thumb, *Rhytidhysteron rufulum* from right lateral malleolus, *Aquastroma magniostiolata* species from subcutaneous cyst on lateral aspect of left lower leg above lateral malleolus, *Aspergillus tamorii*, *Aspergillus glaucus*, *Chetomium* species, *Aspergillus montevidensis*, *Cladosporium sphaerospermum*. Phenotypically unidentified isolates were sent to NCCPF PGI, Chandigarh for final identification.

Conclusion: There is diversity in the etiological agents of subcutaneous mycoses. Every case is different and rare. With the help of molecular techniques, it became possible to identify unusual fungal isolates from subcutaneous infection. Awareness and extensive studies are required to evidence the pathogenicity and associated complication due to these fungal infections. It will also help to regulate the therapeutic management and to know the geographical distribution of unusual fungal agents.

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Pelvic mycoses— an unusual presentation of *Rhizopus arrhizus* in an ommunocompetent patient

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Introduction: The rare but increasingly identified infections caused by Mucorales are always detrimental to the patient due to rapid vascular invasion and the need for thorough surgical debridement and definitive antifungal therapy for its cure. Among the Mucorales, maximum cases have been reported among *Mucor* spp. and *Rhizopus* spp.

Case: Here we present a case of a 9-year-old immunocompetent child presenting with abdominal pain and distension of 1-month duration followed by swelling of bilateral lower limbs, which was gradual in onset. Ultrasonography of the abdomen and pelvis was done which revealed a pelvic mass on the left side. The child developed acute urinary retention during the hospital stay, which was evaluated to reveal a fistulous connection between urethra and rectum for which transverse colostomy was done. The USG-guided biopsy of the left pelvic mass showed broad aseptate hyphae in Hematoxylin and Eosine staining whereas the KOH mount and culture were negative. The follow-up imaging with CT showed circumferential thickening of the recto-sigmoid region and involvement of the posterior bladder wall. Considering the rapidity of the spread of the infection, injection liposomal Amphotericin B at the dose of 5 mg/kg/d, i.v. was started and given for one week with minimal improvement. However, with this clinical picture and patient profile the diagnosis was strongly suspected to be of Basidiobolomycosis of rectosigmoid region. Treatment also was revised to injection of voriconazole at a dose 8 mg/kg/d, i.v. after loading dose which was later shifted to oral dose after 7 days. A repeat USG-guided biopsy was planned for gene sequencing, which identified the organism as *Rhizopus arrhizus*. On follow-up, patient showed no clinico-radiological improvement and in view of the mycological evidence, the antifungal was changed from voriconazole after 2 weeks to oral isavuconazole at the dose of 200 mg/d following the loading after which significant improvement was achieved and patient was discharged.

Conclusion: Mold infection in the form of spreading rectosigmoid mass in an immunocompetent child usually suggests the picture of Basidiobolomycosis. Treating patients only on clinical grounds without mycological confirmation may lead to overlooking of Mucormycosis and may result in adverse outcomes. The diagnosis of Mucormycosis should always be considered as a differential for a fungal infection in the form of mass lesion in abdomen.

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Fungal keratitis caused by *Pseudallescheria boydii*: Clinical and mycological characteristics

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Objectives: *Pseudallescheria boydii* keratitis is rare but important type of fungal keratitis because of the inherently resistance of the organism to many existing antifungal agents. We present the clinical characteristics, risk factors, treatment, and prognosis of patients with *P. boydii* keratitis, and also present the antifungal sensitivities of the isolated strain.

Methods: Slit-lamp and confocal microscopy were used for clinical examinations. Fungal isolates were identified based on morphological characteristics and DNA sequence of the internal transcribed spacer region (ITS). *In vitro* antifungal susceptibility testing for fungal isolates was performed according to the Clinical and Laboratory Standards Institute (CLSI).

Results: *Pseudallescheria boydii* was identified in four patients. All patients had a history of ocular trauma. In clinical examination hypopyon was seen in three patients. The main antifungal medications were oral and topical voriconazole. After treatment the visual activity of all patients improved in 2-3 weeks.

Conclusion: These patients bold the importance of determining causative organism of fungal keratitis and their antibiotic susceptibility. Culture findings are limited in identifying organisms. Sequencing of polymerase chain reaction-amplified DNA is good for accurate and rapid identification of species that can be helpful for optimizing treatment.

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Deep dermatophytosis presented as multiple exophytic masses caused by *Trichophyton* rubrum in immunocompromised patient with rheumatoid arthritis; a case report

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Dermatophyte invades the stratum corneum and infects the skin, nails, and hair, mostly resulting in superficial infection. Deep dermatophytosis involving dermis and subcutaneous layer was rarely reported in immunocompromised state. Herein, we report a case with deep dermatophytosis caused by *Trichophyton (T.) rubrum*. A 71-year-old woman presented with multiple erythematous exophytic and subcutaneous nodules located on both lower legs. She was taking immunosuppressive agents for rheumatoid arthritis and had taken antifungal agents for tinea pedis and onychomycosis, which was improperly ceased. Histopathologic examination revealed pseudoepitheliomatous epidermal hyperplasia with microabscess formation in epidermis and diffuse granulomatous inflammation consisting of multinucleated giant cells, lymphocytes, neutrophils, and histiocytes in dermis. Imuunohistochemical staining with periodic acid-schiff (PAS) and Gomori methenamine silver (GMS) showed septate and branched fungal hyphae in dermis. *Trichophyton rubrum* was identified in fungal culture with tissue and confirmed through phylogenetic analysis of internal transcribed spacer (ITS) and large subunit regions (LSU) in ribosomal RNA. Prior to identification of causative organism, her condition deteriorated into septic shock. Amphotericin B was administered empirically for 6 days in order to prevent hematogenous dissemination and skin lesions were simultaneously resolved. Since deep dermatophytosis appears in various clinical manifestations, it is easy to be mistaken for another disease. If treatment is delayed, immunocompetent patients can progress to severe disease courses like hematogenous dissemination, so clinicians should differentiate this disease and conduct treatment at an appropriate time.

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Epidemiology of *Candida* emia at level-1 trauma care cent er in North India

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Objective: Patients affected by trauma who get admitted to critical care units experience prolonged hospitalization and thereby acquire several infections. This retrospective observational study was done from 2014 to 2021 to observe *Candida*emia affecting this population. A total of 4816 patients admitted with traumatic injuries and hospitalized for treatment at ICU in our 190 bedded Level-1 Trauma center underwent this study.

Methods: Paired blood samples were collected from patients showing signs of sepsis and incubated and monitored regularly by the BacT/ALERT system (bioMe´rieux InC., Marcy l'Etoile, France). All the positive signal samples exhibiting budding yeast cells on Gram stain were subcultured on Chrome agar and Sabouraud dextrose agar. Pure growths obtained were subjected for identification and susceptibility by MALDI-TOF and VITEK 2 system.

Results: Out of the 4816 patients, 61 were affected by *Candida*emia. Out of 61, the maximum was in the age group of 31-40 years (19.7%). Male preponderance (50/61, 82%) was exhibited compared to females. To ascertain *Candida*emia, samples collected were blood (63/66, 95.5%) and CVP tip (3/66, 4.5%). *Candida*emia was primarily observed in patients who suffered major orthopedic trauma (14/61, 21.2%). A total of 66 *Candida* species were isolated from samples of these patients. Out of these, *Candida tropicalis* (43.9%) was the most common, followed by *C. parapsilosis* (22.7%), *C. albicans* (21.2%), *C. haemulonii* (4.5%), *C. glabrata* (3%), *C. rugosa* (3%), and *C. guilliermondii* (1.5%). Concerning antifungal resistance, fluconazole resistance was 16.6% (11), flucytosine 1.5% (1), amphotericin B 6% (4), and micafungin 3% (2). Voriconazole was resistant to none but intermediate to 12.1% (8), caspofungin was intermediate to 3% (2), and resistant to none. A total of 47% (31/61) of patients succumbed to their injuries which were observed highest in the age group of 61-70 years (8/61, 25.8%). The most common injuries that the deceased suffered were polytrauma (9/61, 29%) and blunt trauma abdomen (9/61, 29%). Maximum mortality was also observed in patients with Candidaemia due to *Candida tropicalis* (15/61, 48.3%).

Conclusion: Candidaemia is usually fatal. Mortality due to Candidaemia increases in patients with severe traumatic injuries and added risk factors such as extremes of age, immunocompromised state, and broad-spectrum antibiotics. When compared to a similar study done in our center from 2009 to 2012 (3 years) on Candidaemia in ICU patients, the incidence was lower in our study (12.6% per 1000 ICU admissions vs 14.9% per 1000 ICU admissions), but the mortality rate was higher (47% vs 43.3%). Therefore, a watchful eye on early signs of sepsis, strict hospital infection control measures and antimicrobial stewardship may alter their outcome.