Polyclonal antibodies (PAb) anti-histoplasma were obtained and shown to be reactive against purified H. capsulatum-antigens. Finally, we confirmed the presence of these antigens in yeast culture extracts of H. capsulatum and demonstrated the immunoreactivity of anti-Histoplasma PAb with urine samples from patients previously diagnosed with histoplasmosis.

Conclusion: The generation of novel strategies that combine data analysis, computational tools, and transcriptomic and proteomic techniques could be very useful for the identification of new biomarker genes and the development of microbiological diagnostic tests for important pathogens.

P441

Molecular identification, genotyping, and antifungal susceptibility of *Trichosporon* species isolated from clinical samples of patients at various parts of the Indian subcontinent

Abhila Parashar[1], Vijaylatha Rastogi[1], Hariprashad Prakash[2,3], Shivaprakash M. Rudramurthy[2]
[1]Jawaharlal Nehru Medical College and Associated Hospitals, Ajmer, Ajmer, India
[2]Post Graduate Institute of Medical Education and Research, Chandigarh, India
[3]International Higher School of Medicine, Issyk-Kul Regional campus, Cholpon-Ata, Kyrgyzstan

Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objectives: (1) To study mycological characteristics of strains belonging to *Trichosporon* and its related genera obtained from clinical samples of patients from India. (2) Molecular identification by intergenic spacer (IGS) region 1 sequencing of the rDNA locus. (3) Genotyping of the major causative agent, *T. asahii*, and its *in vitro* drug susceptibility testing.

Materials and Methods: A total of 55 clinical isolates of *Trichosporon* species were collected from NCCPF (National culture collection of pathogenic fungi) PGIMER, Chandigarh along with different health institutions of India. These isolates were recovered from urine, blood, sputum, nail, tissue biopsy, pleural fluid, hair, BAL, and wound discharge over a period of 12 years (2006–2018). The isolates were molecularly characterized and genotyped using IGS-1 region sequencing. *In vitro* drug susceptibility testing of the isolates was performed against amphotericin-B, fluconazole, itraconazole, voriconazole, and posaconazole according to the CLSI M27-A3 guidelines (CLSI 2008).

Results: Predominant underlying risk factors identified were presence of an indwelling catheter, use of broad-spectrum antibiotics, and presence of comorbid conditions such as diabetes, hypertension, and anemia. A total of 47 (85%) of the 55 isolates were identified as *T. asahii*, 6 were *T. inkin* (11%), and 2 were Cutaneo Trichosporon dermatis (3.6%). *Trichosporon asahii* genotype III (22; 41%) was the most common type, followed by genotype IV (12; 22%), I (8; 15%), and VII (2; 4%). In addition to the 15 known *T. asahii* genotypes, one novel genotype was identified in this study. Indian *T. asahii* isolates showed high MIC ranges to amphotericin B (0.06-4 μg/l) and fluconazole (0.25-64 μg/l). Relatively low MIC ranges were found in the case of voriconazole (0.03-1 μg/l), Posaconazole (0.06-1 μg/l), and itraconazole (0.06-1 μg/l). Voriconazole appeared to be the most active drug in maximum *T. asahii* isolates. The MICs for all the drugs were comparatively lower in the case of non-*T. asahii* strains.

Conclusion: *Trichosporon asahii* remains the most common etiology of *Trichosporon*osis in India and presents a challenge for both diagnosis and treatment. With increasing drug resistance, therapeutic options are limited, and antifungal regimens with triazoles especially voriconazole appear to be the best. Accurate timely identification, removal of indwelling catheters/central venous lines, and voriconazole-based treatment along with control of underlying conditions were associated with favorable outcomes. Identification of the novel genotype has epidemiological implications and requires further work up.

P442

Bacterial and fungal infection in COVID-19 diagnosed cases in a tertiary care ICU setting in the wake of second wave in Kolkata, India

Soumyadeep Seal, Dip Narayan Mukherjee
Woodlands Multispecialty Hospital Limited, Kolkata, India

Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Fungal and bacterial infections increase the mortality rate of COVID-19-positive patients. In addition to the risk factors that we cannot change, invasive procedures should be avoided, constant blood sugar regulation should be applied, and unnecessary antibiotics use should be avoided.

To investigate the incidence of bacterial and fungal infection of hospitalized patients intensive care units with confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in this retrospective observational study in a tertiary care hospital in Kolkata in the wake of second-wave in India.

A retrospective study of hospitalized patients with confirmed SARS-CoV-2 by PCR was analyzed study in a tertiary care hospital in Kolkata in the wake of second-wave from February 2021 to October 2021.

The records of 327 patients hospitalized in ICU with the diagnosis of COVID-19 were investigated from electronic health records and hospitalization files.

The demographic characteristics (age, gender), the number of ICU hospitalization days and mortality rates, APACHE II scores, accompanying diseases, antibiotic-steroid treatments taken during hospitalization, and microbiological results (blood, urine, tracheal aspirate samples) of the patients were recorded. Blood cultures, respiratory samples, pneumococcal or Legionella urinary antigens, and respiratory viral PCR panels were obtained from COVID-19 patients, respectively. The average APACHE II score of the patients was 28 ± 6.

A positive blood culture was identified in 60 patients (7.1%), of which 39 were classified as contaminants. Bacteremia resulting from respiratory infection was confirmed in two cases (one each community-acquired Klebsiella pneumoniae and ventilator-associated Enterobacter cloacae). Line-related bacteremia was identified in six patients (three *Candida*, two *Enterococcus* spp., and one *Pseudomonas aeruginosa*). All other community-acquired bacteremias ($n = 16$) were attributed to non-respiratory infections. Zero concomitant pneumococcal, Legionella or influenza infection was detected. A low yield of positive respiratory cultures was identified; Staphylococcus aureus was the most common respiratory pathogen isolated in community-acquired coinfection (4/24; 16.7%), with pseudomonas and yeast identified in late-onset infection. Invasive fungal infections ($n = 3$) were attributed to line-related infections. Opportunistic fungal infection was detected in 58 patients (17.37%) of 327 patients monitored in ICU with a COVID-19-positive diagnosis. *Candida albicans* was the opportunistic fungal agent isolated from most blood samples taken from COVID-19-positive patients. The mortality rate of COVID-19-positive patients with candidemia was 80%. While 2/3 patients (66.6%) for whom fungi were grown from their tracheal aspirate died, one patient (33.3%) was transferred to the ward.

Prolonged mechanical ventilation support was associated with the development of nosocomial candidemia and bacteremia. Parallel to the developments in the field of diagnosis and treatment, an increase in the incidence of fungal infections and the number of patients who are in the risk group for the development of opportunistic fungal infections have been observed in recent years. Among the hospitalized patients, those most at risk in terms of fungal infections are intensive care unit (ICU) patients. The rate of *Candida* infections amongst critical care patients is very and may pose severe mortality if not diagnosed, treated, and handled effectively, and promptly.

P443

Altered expression of fungal CotH, human glucose-regulated protein 78 (GRP78), and predicted miRNAs in macrophages and model diabetic mice infected with *Rhizopus oryzae*

Tahereh Shokohi[1], Zahra Seifi[2,3], Mohammad Shafie[3,4], Javad Mowla[5], Farhad Niknejad[3,6], Mahdi Abastabar[1], Lale Vahedi Larijan[7]
[1]Invasive Fungi Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran, Sari, Iran
[2]Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran, Sari, Iran
[3]Department of Laboratory Sciences, Faculty of Paramedicine, Gorgan University of Medical Sciences, Gorgan, Iran, Gorgan, Iran
[4]Golestan Research Center of Gastroenterology and Hepatology, Department of Human Genetics, Golestan University of Medical Sciences, Gorgan, Iran, Gorgan, Iran
[5]Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran, Tehran, Iran
[6]Laboratory Sciences Research center, Golestan University of Medical Sciences, Gorgan, Iran, Gorgan, Iran
[7]Gastrointestinal Cancer Research Center, Non-communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran

Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objectives: *Rhizopus oryzae* is one of the most common causes of mucormycosis. Among the virulence factors of the Mucorales, CotH protein has recently been identified, which causes the invasion of *R. oryzae* into endothelial cells. In this study, we aimed to examine the reaction between GRP78 at the level of human cells and different groups of mice and CotH3 at the surface of the *R. oryzae* hyphae. We evaluated the relative expression of GRP78 and CotH3 genes and changes in the expression of some miRNAs that target the human GRP78 gene.

Methods: In this study, the relative changes in gene expression were studied. In three groups (1) Macrophages derived from human monocytes: monocytes from the blood of healthy donors were isolated using Ficoll and in RPMI 1640 medium containing FCS 10% and with penicillin-streptomycin after 2 weeks were differentiated into macrophages. Two groups were investigated, including control and infected with *R. oryzae* hyphae, for 6 and 16 hours after infection. (2) Hematogenous dissemination mucormycosis model: Seven groups of male BALb/c mice were examined in control, infected, and treated groups with Liposomal amphotericin B. (3) Human mucormycosis: in this study, two samples of patients with rhinocerebral mucormycosis with diabetes mellitus treated and untreated with liposomal amphotericin B were examined. Total RNA extraction and cDNA synthesis were performed from the studied samples. The relative expression changes of the target genes and miRNAs were evaluated using real-time PCR carried out using Sybrgreen-based detection methods.

Results: Monocyte-derived macrophages had a steady pattern in relative changes in gene expression. An increase in expression of two genes, GRP78 and CotH3, was observed in the samples, and all miRNAs targeted by the GRP78 gene included hsa -miR-16-5p; hsa -miR-335-5p and hsa -miR-93-3p showed a decreasing pattern. In the mice mucormycosis model, relative gene expression changes were observed, and mmu-miR-181b-5p showed increased expression deviation in all groups. The clinical sample of diabetic patients with untreated rhinocerebral mucormycosis also had a consistent pattern of GRP78 and CotH3 increased gene expression. The hsa-miR-16-5p and hsa-miR-335-5p have increased expression, while hsa-miR-93-3p decreased expression.

Conclusion: After validation, these micro RNAs can be used as valuable markers in mucormycosis detection and treatment processes.

P444

Detection of causative agents of infectious keratitis in patients from western rajasthan

Twishi Shrimali[1], Anuradha Sharma[1], Anup Ghosh[2], Arvind Morya[1], Vibhor Tak[1], Vijayalakshmi Nag[1]
[1]All India Institute of Medical Sciences Jodhpur, Jodhpur, India
[2]Post Graduate Institute of Medical Education and Research, Chandigarh, India

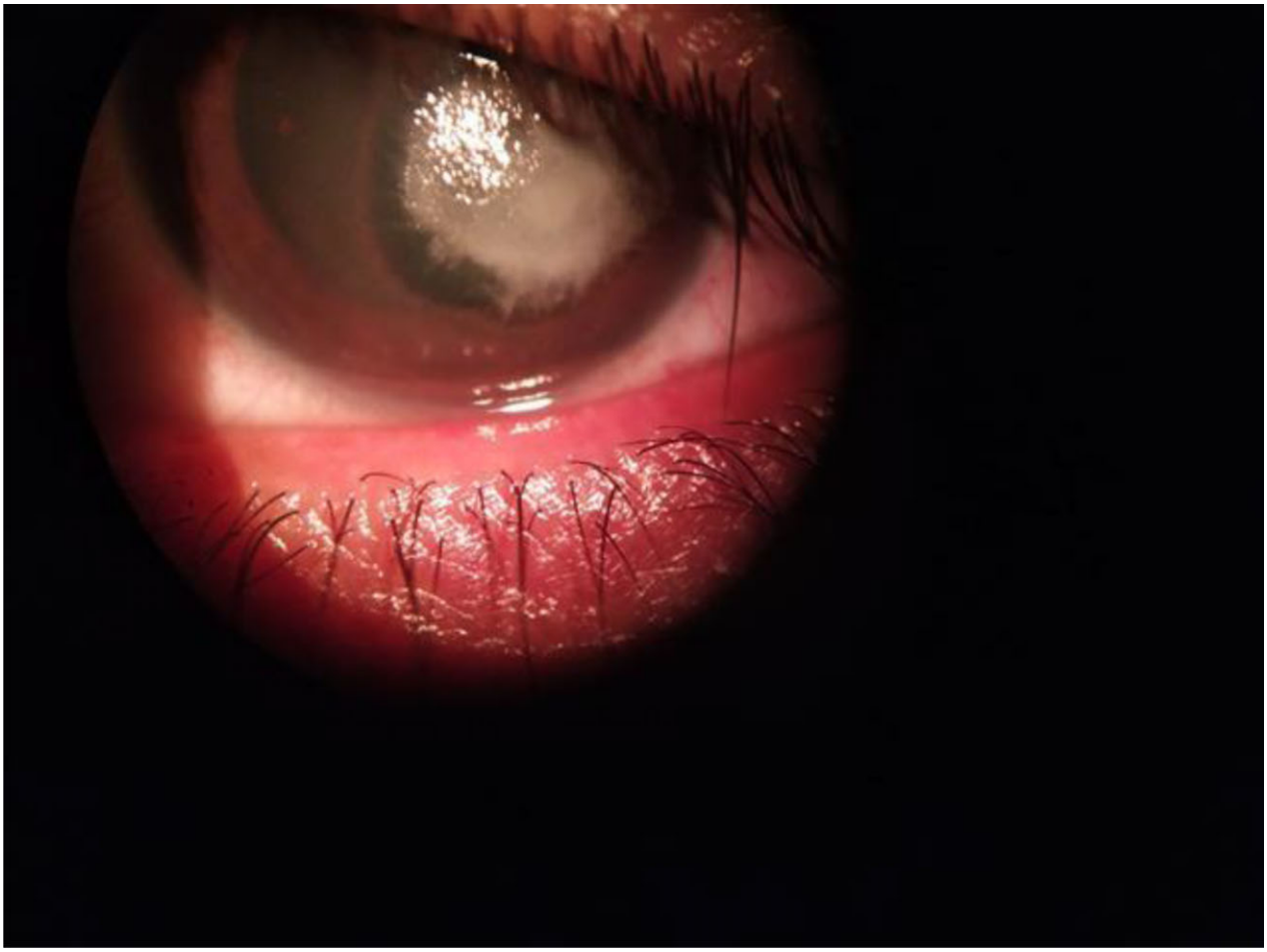
Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objective: To determine the spectrum of causative agents, the related risk factors, and their association in patients of infectious keratitis.

Methodology: It was a prospective study conducted over a period of 18 months from August 2018 to January 2020, which included 100 patients attending the Ophthalmology OPD with features of keratitis. Ophthalmological examination was followed by corneal scrapings' collection, which were subjected to culture, microscopy, and molecular diagnostic tools. Bacterial isolates were identified by conventional methods and MicroScan Walkaway system while the isolated fungi were identified conventionally. Pan-fungal primers were used to detect fungal elements directly from the sample.

Results: Out of 100, 41 cases were positive by culture, of which 32 (78.04%) had fungal and nine (21.95%) had bacterial keratitis. *Fusarium* spp. accounted for 33.33% of fungal and *Pseudomonas aeruginosa* accounted for 55.55% of the bacterial isolates. Fungal material was detected in 41% using pan fungal primers. Cases were maximally recorded during July-October. Traumatic history was present in 78% patients caused by vegetative matter (49%). A male preponderance (67%) was also observed. Four patients underwent evisceration in spite-of rigorous management.

Conclusion: Poor prognosis emphasizes the need for faster diagnostics, which can detect the causative agents from the clinical specimen itself, reinforcing the concept of clinical metagenomics.



P445

Clinical evaluation of the performance of the most commonly used eumycetoma diagnostic tests using sequencing of the internally transcribed spacer region as the golden standard

Emmanuel Siddig[1], Bertrand Nyuykonge[2], Najwa Mhmoud[1], Omnia Abdallah[1], Mustafa Bahar[1], Eiman Ahmed[1], Borna Nyaoke[3], Eduard Zijlstra[3], Annelies Verbon[2], Sahar Bakhiet[1], Ahmed Fahal[1], Wendy van de Sande[2]
[1]University of Khartoum, Khartoum, Sudan
[2]Erasmus MC, University Medical Centre Rotterdam, Department of Medical Microbiology and Infectious Diseases, Rotterdam, Netherlands., Rotterdam, The Netherland
[3]Drugs for Neglected Tropical Disease Initiative, Switzerland

Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objective: Mycetoma is a neglected tropical skin disease, caused by 70 different causative agents. For most of the causative agents, molecular identification is the only reliable method to identify the species level. In practice, ultrasound, histopathology, culturing, and species-specific PCRs are most commonly used for species identification. However, the performance of these different tests was not validated using molecular identification by sequencing barcoding genes.

Methods: In this study, we validated the performance of the most commonly used diagnostic tools including culture, histopathology, Ultrasound and two species-specific PCR for *Madurella mycetomatis* on 222 patients suspected of fungal mycetoma by *M. mycetomatis*; the sensitivity, specificity, and accuracy of each method was calculated.

Results: From the 222 patients, 154 (69.3%) were correctly identified by ultrasound, histology, culture, and both species-specific PCRs. For five patients all tests were negative and for three only the ultrasound was indicative of mycetoma. For the other 60 patients, at least one of the assays was negative for *M. mycetomatis*. The two species-specific PCRs were the most sensitive and specific, followed by culture and histology. Ultrasound was the least specific as it only allows to differentiate between actinomycetoma and eumycetoma. However, with ultrasound, an identification could be obtained in 9.38 min. PCR took 3.76 h, histology 8.5 days, and culturing 21 days.

Conclusion: We concluded that PCR directly on DNA isolated from grains is the most rapid and reliable diagnostic tool to identify M. mycetomatis from eumycetoma grains to use species-specific PCRs. In order to shorten the time to identification of other causative agents, the focus should be on developing more molecular assays for those species.

P446

Molecular detection of fungal agents responsible for COVID-19-associated mycosis directly from tissue specimens

Vikramjeet Singh[1], Anupam Das[1], Ashish Chandra Agarwal[2], Nikhil Gupta[3], Manodeep Sen[1], Jyotsna Agarwal[1]
[1]Department of Microbiology Dr Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Lucknow, India
[2]Department of Otorhinolaryngology Dr Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India
[3]Department of General Medicine Dr Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India

Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objective: Due to the ongoing COVID-19 pandemic a new group of patients at risk emerged with COVID-19-associated mucormycosis (CAM) and other fungal infections. Molecular studies, evaluating the prevalence of CAM and other fungal infections are lacking. To assess CAM prevalence in a super-specialty healthcare hospital in North India, we applied direct microscopy, fungal culture, and qualitative real-time-PCR targeting Mucorales-specific fragments on tissue specimens of critically ill COVID-19 patients.

Methods: This was a hospital-based prospective study during second-wave of COVID-19 in India. All clinically suspected CAM patients with a history of COVID-19 were included in the study from March 2021 to June 2021 where tissue or biopsy specimens were collected under aseptic conditions. Conventional identification methods were performed for all isolates, speciation was done by MALDI-TOF, and comparative detection by RTPCR was also done.

Results: In the present study, among 67 samples received in the laboratory from clinically suspected CAM patients, 32 samples showed positive growth using the conventional method of identification. *Rhizopus arrhizus* was the commonest fungal isolate obtained followed by *Aspergillus flavus* from tissue samples. Use of molecular and automated machines helped in the early identification of these species 24-48 h less than the conventional methods. Polyfungal isolates are also reported from two tissue samples of patients in the post-COVID discharge stage. Almost 90% of patients with CAM and other fungal etiology agreed to steroid intake and diabetes condition during COVID-19 infection.

Conclusion: Considering the ever-evolving strains and variants of COVID-19, it is important to have a high index of suspicion for fungal coinfection in patients with COVID-19 presenting with comorbidities. Further, they should undergo immediate molecular studies with an emphasis on the requirement of medical or surgical intervention if the result comes positive. There is a need to stress on the judicious use of steroids to avoid flaring up of the fungal infection.