

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.

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

Abhila Parashar¹, Vijaylatha Rastogi¹, Hariprakash Prakash^{2,3}, Shivaprakash M. Rudramurthy²

¹Jawaharlal Nehru Medical College and Associated Hospitals, Ajmer, Ajmer, India

²Post Graduate Institute of Medical Education and Research, Chandigarh, India

³National Higher School of Medicine, Issyk-Kul Regional campus, Cholpon-Ata, Kyrgyzstan

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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 Cutaneous *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 *Trichosporonosis* 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.

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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

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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.

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

Tahereh Shokohi¹, Zahra Seifi^{2,3}, Mohammad Shafie^{3,4}, Javad Mowla⁵, Farhad Niknejad^{3,6}, Mahdi Abastabar¹, Lale Vahedi Larijan⁷

¹Invasive Fungi Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran, Sari, Iran

²Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran, Sari, Iran

³Department of Laboratory Sciences, Faculty of Paramedicine, Gorgan University of Medical Sciences, Gorgan, Iran, Gorgan, Iran

⁴Golestan Research Center of Gastroenterology and Hepatology, Department of Human Genetics, Golestan University of Medical Sciences, Gorgan, Iran, Gorgan, Iran

⁵Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran, Tehran, Iran

⁶Laboratory Sciences Research center, Golestan University of Medical Sciences, Gorgan, Iran, Gorgan, Iran

⁷Gastrointestinal Cancer Research Center, Non-communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran

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Objectives: *Rhizopus oryzae* is one of the most common causes of mucormycosis. Among the virulence factors of the Mucorales, CoH 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 CoH3 at the surface of the *R. oryzae* hyphae. We evaluated the relative expression of GRP78 and CoH3 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 CoH3, 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 CoH3 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.

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

Twishi Shirmali¹, Anuradha Sharma¹, Anup Ghosh², Arvind Morya¹, Vibhor Tak¹, Vijayalakshmi Nag¹

¹All India Institute of Medical Sciences Jodhpur, Jodhpur, India

²Post Graduate Institute of Medical Education and Research, Chandigarh, India

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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.