Issue 22-December 2020

ISMM MYCOSES Newsletter

Report of President

Dear Friends ,

Wishing you all a Covid free new year ! It's been a long gap since we have met each other in person since all the Mycology programs are happening in a virtual platform. We successfully conducted the online 13th Annual Basic and Molecular Mycology Workshop from 24th to 27th of November 2020.

Last year has been a year of happiness and sadness in many ways. The demise of Dr. Randhawa has come as a great loss to the Mycology fraternity. He has contributed immensely to the field of Mycology. I had met him few times in some of the conferences . I remember him as a very simple and unassuming person.

The happy moment was when two of our Eminent Mycologists were recognised as top 2 % of world scientists according to a Stanford Study. Dr. Arunaloke Chakrabarti and Dr. Savitri Sharma. They deserve it. We are very proud of this recognition given to scientists from India.

The resurgence of fungal infections in post covid patients has increased and we have to be alert about it. Dr. Atul Patel under the leadership of Dr. Chakrbarti has initiated a study on the increase in zyogmycetes cases among post Covid patients.

The ISHAM conference has been rescheduled to 8th March to 12th March 2022. I request all to please become members of ISHAM at the earliest to avail of all the benefits offered by the Society. There is a provision for joining the young ISHAM group for those whose age is 40 years or less.

The ball has started rolling once again for the organising team and the members of various committees. I expect youngsters in large numbers to attend the conference and present their papers.

I really thank Dr. Jayanthi Savio for the immense effort she has taken to start the ISMM Website.

I hope it will come up well with all the updated information on events and happenings.

Once again, wishing all a very happy and prosperous, academically active new year.

Dr. Anupma Jyoti Kindo
President, Indian Society of Medical Mycologists

Report of General Secretary

Dear ISMM family members,

Here's wishing you all a wonderful, peaceful, healthy 2021.

I know all of us went through one of the stress full years of our lives with the pandemic. But I'm sure we all did well to contribute to the society in our own ways and we have to congratulate ourselves for that.

Last year we have lost some eminent microbiologists. With passing away of Professor Dr. Harbans Singh Randhawa [1933- 2020] Indian microbiologists and mycologists in particular have lost an eminent teacher, researcher, visionary and guide. His passion for mycology began with his work on M.Sc. thesis. He served as the Professor & Head, Medical Mycology Unit, Dept. of Microbiology and ex-Director of Vallabhbhai Patel Chest Institute (VPCI) (1991-1998). He served in various capacities and was awarded several national and international research fellowships. He mentored many research scholars and had over 180 publications. He served as Vice-President of the International Society for Human and Animal Mycology for 3 years (1985-1988). His passing away has definitely closed a big chapter in medical mycology in India. The members of ISMM are with his family and friends in this time of sorrow. [Visit the ISMM home page on more details and messages]

Though the last year was a challenge in terms of conducting academic programs, some of our colleagues did conduct successful programs online in mycology. I congratulate Dr. Reba Kanungo for conducting the CME and our President Dr. Anupma Lakra for conducting the five days workshop in her own inimitable style.

As I did predict in my last secretary's report, we are now seeing a surge in invasive fungal infections. Working together we can bring out some interesting data.

The new website is launched. [www.ismm.in]. It is still very rudimentary. I urge members to log in. Please send in your feedback. We will make the changes so that it is vibrant and user friendly.

A special thanks to Dr. Anuradha Sharma [AIIMS Jodhpur] for her contribution of 2 lakhs from the proceeds of the 13th National Congress of our Society she conducted. The members of the society and I in particular appreciate her generosity.

I am happy to keep you all informed that the 21st Congress of ISHAM will be held from 8th -12th March 2022 at New Delhi. We shall work as a team led by Dr. Chakrabarti and make this an event to remember.

Once again many a thanks to Dr. Savitri Sharma for her patience with all of us and her commitment to get the newsletter published on time.

Friends, prayers and best wishes to each one of you and your loved ones for a great year ahead.

Stay safe and stay healthy.

Jayanthi Savio
General Secretary, SIHAM

1. **Dr. M. J. Thirumalachar Life Time Achievement Award.**

The Life Time Achievement award is established to honor members of the Society, who during the span of his/her lifetime have demonstrated a longstanding commitment to the cause of Medical Mycology in India. The award is made possible by a generous donation by one of the senior most and revered member of the Society, Dr. Arvind A. Padhye,

The award would recognize the significant contribution to the understanding and application of the knowledge pertaining to the Medical Mycology in India, over the entire course of his /her life time, with a definable body of work through one or more of the following:-

- Teaching /Training.
- Research.
- Publications/patents.
- Patient care.

Who may receive the award?

The nominee should be a Life member of the Society in good standing,

He should be in the field for at least 25 years but not necessarily active professionally at the time of receiving the award.

He must be alive at the time the selection committee's choice is announced. In case of an unfortunate event of death of the awardee after selection, the award may be presented posthumously.

How will the recipients be chosen?

The president, with the approval of the executive committee, will appoint a Life Time Achievement Awards committee consisting of five active members of the Society. One committee member shall be a current member of the SIHAM executive council, who would co-ordinate the committee meeting. The committee will invite nominations from the members for the award. The nomination is to be made by at least two life members of the society at least 6 months in advance to the next annual conference of the society. Self-Nomination will not be accepted.

The nominations will be scrutinized by the award committee and the best among the nominations will be selected for the award.

When will the award be presented?

The award may be presented to the deserving individual at the Annual Conference of the Society. The awardee will be introduced to the august gathering duly stating his/her achievements during the inaugural function of the Conference.

The award will consist of a citation and a memento.

No travelling or daily allowance will be provided to the awardee to attend the function.

The decision of the award committee will be final.

2. **G. P. Agarwal young scientist Award**

The best paper award will be given to a young scientist below the age of 35 years (proof of age to be submitted). Applicant must submit the full length original research paper on any area of the medical mycology. Oral presentation of the research should be done in the separate award session during the conference.

3. **Dr. Pankajalakshmi Venugopal Glaxo Meritorious Award**

Age limit -35 years (proof of age to be submitted). Must submit the curriculum vitae with list of publications and reprints of the papers in the field of medical mycology. Award will be given on the basis of the CV for the outstanding work in the field of medical mycology

4. **Dr Kamalam Glaxo award**

Applicant must submit full length research paper in duplicate in the field of dermatomycology. Award will be given based on oral presentation in the separate session during the conference.

Invasive Aspergillosis in COVID 19 non intubated patient

[1]Chhavi Gupta, [1]Sayed Hassan, [1]Anjani Kumar Tiwari, [1]Sanjay Joshi, [2]*Shukla Das

[1]Department of Medicine; Northern Railway Central Hospital, New Delhi, India

[2]Department of Microbiology, University College of Medical Sciences, Delhi, India

*Corresponding author (Email: shukladas_123@yahoo.com)

Introduction:

Invasive Aspergillosis (IA) typically known to occurs in the setting of severely immunosuppressed hosts. Recently, viruses particularly Influenza virus, Human Cytomegalovirus, Respiratory Syncytial Virus have been identified as associated emerging risk factor. Influenza associated invasive Aspergillosis (IAIA) was rarely diagnosed before 2009 HINI pandemic, but thereafter it has become a well-recognized entity. The impaired host adaptive immune response is considered as important factor for IAIA and if left untreated, mortality rates tend to be high. Paralleling with IAIA, COVID associated pulmonary Aspergillosis (CAPA) has been identified as a distinct entity. There is wide variation in the reported incidence of CAPA ranging from 4% to 35% in mechanically ventilated patients. The diagnosis remains challenging due to non-specific and overlapping radiology of CAPA and COVID 19 pneumonia and ARDS and further due to high risk of exposure, difficulty in performing bronchoscopy and computed tomography (CT) scans. We report a case of invasive fungal disease (IFD) in non-intubated non immunosuppressed COVID 19 patient.

The time to detection of IA in COVID 19 patients post admission to the ICU ranged from 0 to 35 days with median of 8 days. All these cases have been reported in mechanically ventilated patients. In our case IA was diagnosed after 14 days of ICU admission in non-intubated patient.

Case report:

A 58-year-old man with comorbidities of hypertension and diabetes mellitus and history of interstitial lung disease with previously treated pulmonary tuberculosis presented with history of fever and shortness of breath for two days. On admission, his vitals were blood pressure (BP) -130/80 milimetere (mm) of mercury, pulse rate (PR)- 78 beats per minute, respiratory rate (RR) – 24 per minute, and oxygen saturation of 94 % on 6 litres of oxygen per litre, temperature 101 degree celsius. Initial chest X ray (CXR) showed bilateral peripheral opacities (Fig. 1). SARS-CoV-2 RNA was detected in nasopharyngeal and throat swab by reverse transcriptase polymerase chain reaction (RT-PCR) method. He was started on injection remdesivir for 5 days, tablet doxycycline 100 mg twice daily and other supportive treatment (methylprednisolone and low molecular weight heparin). Within next 3-4 days his condition deteriorated with increasing oxygen requirements of 16 litre/minute via NRBM, with further worsening he was put on high flow nasal cannula (HFNC) with fraction inspired oxygen (Fio2) of 40% at 40 litre/minute. On 6th day his TLC increased to 17000 /mm3 which further escalated to 22000/mm3, with increasing count he was initiated on broad spectrum antibiotics, his blood culture and urine cultures were sterile. Gradually around

10th day, his oxygen requirement was reduced to 20 litre/minute and CXR shows improvement (Fig. 2), fever subsided with TLC showing declining trend. However, on day 14 of admission, his oxygen requirement again increased with 40 litres/min via HFNC . TLC again rose to 26000/mm3, his COVID RT PCR was still positive on day 15. Even after 10 days of antibiotics after initial improvement, his counts were persistently high and with high oxygen requirements about 60 litre/minute via HFNC. The urine culture and blood cultures were sterile. In view of persistently high white blood cell counts and high oxygen requirements, progressively increasing infiltrates on CXR (Fig. 3), invasive fungal disease (IFD) was thought of. Patient was not able to expectorate sputum, his serum Galactomannan (GM) was sent for. CT scan and bronchoscopic studies were not done to avoid risk of exposure. The serum GM was reported to be 0.97 (cut off 0.5) and diagnosis of IA was made. The patient was started on oral voriconazole (6 mg/kg loading followed by 4 mg/kg twice daily) on day 18 of admission. There was rapid clinical and radiological improvement over the next 7 days (Fig. 4). TLC settled, oxygen requirements gradually reduced to 30 litre/min via HFNO with resolution of infiltrates on chest radiology, ABG pH – 7.4, Po2 – 115, PaCo2 – 45, Hco3 – 29.4 . After 1 month his RT PCR came negative and he was shifted to non COVID ICU on 15 litre oxygen per litre.

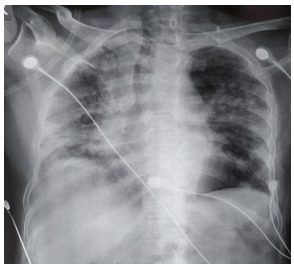


Figure 1: Chest radiograph on day of admission, showing poorly defined pulmonary opacities bilaterally, located principally in the middle and lower lung fields.

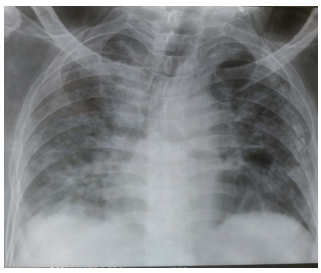


Figure 2: Chest radiograph day 10 of admission initial improvement of bilateral opacities.

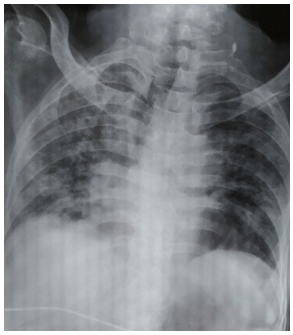


Figure 3: Chest radiograph on day 14 showing increased confluent right lung opacities and persistent focal opacities in left lung.

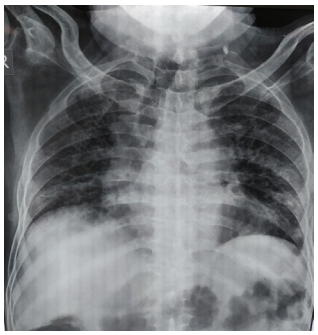


Figure 4: Voriconazole treatment day 7 showing overall improvement.

Discussion:

The diagnosis of IA is relied upon European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) which has been validated in immunocompromised patients. Given high risk of exposure, bronchoscopic studies and CT scans are not always feasible in COVID 19 infections. Various reports have utilized different diagnostic criteria for diagnosis of CAPA but no validated algorithm has been proposed. For decades now, fungal biomarkers; Galactomannan (GM) and Beta- D glucan (BDG) have been used for IFD especially in transplant or oncology settings. It is believed that these biomarkers are detectable even before clinical symptoms start and hence are used for pre-emptive treatment. GM is the carbohydrate constituent of the cell wall of *Aspergillus* spp., released by the fungus during cell growth. The GM cut off in serum is 0.5, the sensitivity is poor in non immunocompromised patients due to robust immune response, poor fungal burden, less dissemination and release. In our patient, we used the recently proposed definitions of IAIA which includes non-specific radiology and single positive serum GM for making diagnosis and the patient responded well to antifungal therapy. We believe that high suspicious is required in COVID 19 patients even in absence of mechanical ventilation and preemptive therapy should be initiated as early as possible. There are still several unanswered questions like timing of presentation, screening protocol, diagnostic criteria and treatment guidelines which can be addressed by large studies.

Clinicomycological Study of *Candida* Isolates in a Tertiary Care Hospital- A Pilot Study

Shibani Mishra, Anitha Subramanium, *Anupma jyoti Kindo

[1] Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Porur, Chennai – 600116, India.

* Corresponding author (anupmalakra@gmail.com)

Introduction

Candida is an opportunistic fungus, which can be found as a part of normal flora in the body. One hundred and fifty different species of *Candida* are known out of which *C.albicans, C. tropicalis, C.parapsilosis, C.krusei, C.glabrata* are medically important [1]. *C. tropicalis* is the most common type of *Candida* in India [2]. *Candida* usually causes muco-cutaneous infections but if severe it can cause invasive disease, which is known as candidemia. *Candida* can be found in the oral cavity of at least 75% of the population and it is seen that about 70% of women suffer from vulvovaginal candidiasis [3]. It causes 8% of all infections and is the fourth most common cause of blood stream infection [4]. Due to its property to be able to switch between the yeast and hyphal form, it is able to survive and adapt to different temperatures, form biofilms and cause disseminated disease [5]. Although *Candida* is a commensal organism and is seen in various parts of a healthy individual it becomes pathogenic in states of immunosuppression like AIDS, pregnancy, patients undergoing surgery, organ transplantation, and patients on chemotherapy [6]. Metabolic conditions like diabetes and injudicious usage of high dose antibiotics and antifungals increase the chances of acquiring *Candida*. It is seen that one of the major causes of an increase in non- albicans *Candida* is the indiscriminate use of azoles [6]. Since identifying the species of *Candida* quickly and effectively is necessary to be able to start the appropriate antifungal, in this study we use PCR-RFLP to speciate *Candida*. The study was also done to analyze the correlation of isolates with the patient's clinical condition and to study the outcome of the patient.

Materials and Methods

A prospective cross sectional study was carried out for a period of 2 months at the department of Microbiology, Sri Ramachandra Institute of Higher Education and Research, Chennai- a tertiary care center. Twenty-five clinical isolates of *Candida* were taken up for the study. ATCC *C. albicans* 90028 was included as standard control strain.

DNA extraction

DNA was extracted from all clinical isolates by phenol-chloroform method. *Candida* colonies were suspended in 500 µL of lysis buffer

(10mM TRIS, pH - 8), 1mM EDTA (pH - 8), 3% SDS and 100 mM NaCl) and kept in the water bath at 100ºCfor 2 minutes. Equal volume of Phenol: Chloroform (500µL) was added to it and mixed well. It was centrifuged at 10,000 rpm for 5 min. The aqueous layer was then transferred to a fresh microcentrifuge tube; 500µL of chloroform was added and was centrifuged at 10,000rpm for 5 mins. DNA precipitation was done by adding an equal amount of isopropyl alcohol, which was centrifuged and then washed with 300µL of 70% ethanol. Then 50µl of TE buffer was added and stored at -20ºC.

PCR assay

The master mix was prepared containing 10 µL of PCR mix, 0.5 µL of forward (ITS-1) and reverse primer (ITS-4) (Sigma), 2 µL of template DNA and 7µL of sterile nuclease free water, total volume of the mixture being 20 µL. This was then subjected to polymerase chain reaction (PCR): initial denaturation at 95ºC for 5 min, denaturation at 95ºC for 30 sec, annealing at 56 ºC for 30 sec, extension at 72 ºC for 30 sec and final extension at 72 ºC for 5 min. PCR products were electrophoresed in 1.5% agarose and visualized under trans- UV illumination.

RFLP

Two µL of enzyme buffer, 0.5µL of Msp I (GeNei, Bangalore) enzyme, 7.5µL of nuclease free sterile water and 10 µL of PCR product were added in a 200-µL PCR tube and it was incubated at 37ºC for 2hrs. RFLP products were electrophoresed at 2% agarose gel.

Patient details such as demographic details, diagnosis on admission, duration of stay in the hospital, course in the hospital, medications (antibiotics/ antifungal usage), culture reports, covid19 status, and underlying illnesses were collected from the hospital discharge summary and the lab reports and the patients were followed up until discharge from the hospital.

Results:

Among the 25 samples that were collected over two months (August and September 2020), 6 species of *Candida* were identified using PCR-RFLP (Fig. 1, Fig. 2), they were- *C. tropicalis* 14 (56%), *C. albicans* 5 (20%), *C. auris* 3 (14%), *C. parapsilosis* 1 (4%), *C. orthopsilosis* 1 (4%), *C. kefyr* 1 (4%). 17 (68%) of them male and 8 (32%) of them were female. Out of the 25 samples collected- 15 (60%) of them were urine samples, 8(32%) were blood samples, 1(4%) was pus sample and 1(4%) was tissue sample.

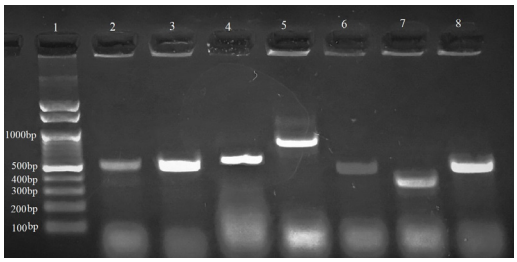


Figure 1: PCR products of representative *Candida* species: Lane 1-8 (DNA ladder, *C. albicans, C. tropicalis, C. papapsilosis, C. kefyr, C. orthopsilosis, C. auris,* ATCC C. *albicans*

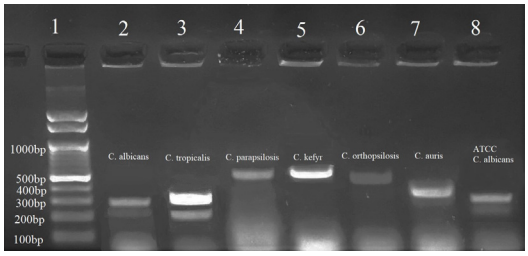


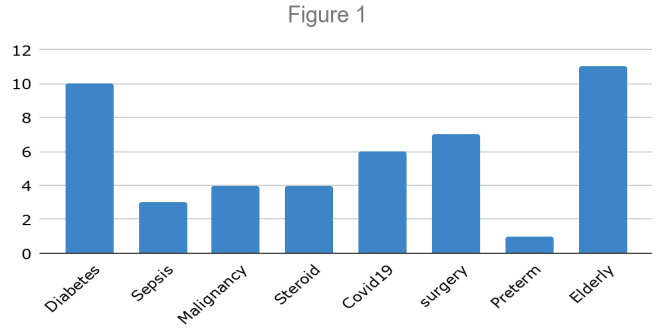
Figure 2: RFLP products of representative *Candida* species

The average duration of hospital stay was 13.8 days (1-34 days). The age of the patients ranged from preterm of 32 weeks to 80 years. The underlying risk factors that were present are shown in Fig. 3. Apart from these, there were other underlying comorbidities such as- Hypertension in 5 (11%) patients, Coronary artery disease in 4 (9%) of patients. Chronic Kidney Disease in 2 (4%) of patients, Acute kidney injury in 1 (2%) of patients. Chronic liver disease was in 1(2%) patient, cerebrovascular disease in 1(2%) patient, Tuberculosis in 1(2%) patient. Among 25 patients 6(13%) patients were covid19 positive. Table 1 shows the sample and species of *Candida* isolated from them. Among 25 patients 18 (72%) of them were discharged, 4 (16%) of them refused treatment against medical advice and 3 (12%) of them died.

Table 1- Underlying illness in patients with Covid-19 and their samples and isolates

Underlying illness in patients with Covid-19	Sample	Isolate
Diabetes Mellitus, invasive entilation	blood	C. tropicalis
Diabetes Mellitus, disseminated bacteremia	urine	C. tropicalis
Diabetes Mellitus	urine	C. tropicalis
Diabetes Mellitus	urine	C. tropicalis
Diabetes Mellitus	urine	C. tropicalis
None	urine	C. tropicalis

Figure 3- Underlying risk factors present in patients



Discussion:

Candidemia is one of the leading causes of mortality in today's world and the need to be able to speciate *Candida* accurately and rapidly has become all the more necessary. In this study, PCR- RFLP was done to speciate *Candida* since it is a rapid and relatively cheaper method of correctly identifying the species.

Out of the 25 isolates, *C. tropicalis* was the most prevalent (56%) followed by, *C. albicans* 5(20%), *C. auris* 3(14%), *C.parapsilosis* 1(4%), *C. orthopsilosis* 1(4%), *C. kefyr* 1(4%) This shows an emergence of non-albicans species. *C. tropicalis* has become the most common species of *Candida* among the Indian hospital statistics. *C. auris* has become a pandemic ever since its isolation in Japan [7]. It has also become quite common in patients admitted in hospitals, especially in ICUs in India [8]. *C. auris* isolates in our study were blood samples. These patients had severe illness with underlying risk factors and one of the patients succumbed to the illness. It is now a known fact that *C. auris* is resistant to the commonly used antifungal agents like azoles and amphotericin B [9]. Some of the isolates may even be resistant to

the echinocandins like caspofungin, micafungin and anidulafungin [10]. In our study there was one *C. kefyr from* a patient who had acute pancreatitis (sample was taken from blood); *C. kefyr* usually causes disseminated candidiasis particularly in patients who have hemato-oncological malignancies [11].

Multiple risk factors were present in our patients, which included an increased hospital stay, antibiotic usage, and multiple catheterizations. Our study showed that the average duration of hospital stay was 14 days as compared to another study conducted in India by Chakrabarti et al. in which the duration of hospital stay was 9 days [8].

During the corona spread 6 patients had covid-19 infection and all of them had Diabetes Mellitus. Since most of them were urine samples, it cannot be ascertained if they were due to the infection per se. We could not establish them being disseminated as none of the blood isolates of these patients grew *Candida*.

Seven of our patients had undergone various surgical procedures including major surgeries like appendicular abscess drainage, debulking surgery for carcinoma rectosigmoid, open abdominal surgery for necrotizing enterocolitis and pancreatitis, debulking surgery for pseudomyxoma peritonei, and repair of the urinary bladder and sigmoid colon rupture due to traumatic injury. Many studies show that abdominal surgeries carry the highest risk factor amongst different surgeries for causing disseminated Candida infection [8].

In our study 16 patients were on long-term antibiotic therapy like meropenem, piperacillin and tazobactam. A number of studies show that this is a common and independent risk factor associated with candidemia, this is consistent with a study conducted by Xess et al. 2007 [12] and Giri et al. 2013 [13] in tertiary care centers in India.

Conclusion:

Since the outcome of the patient depends upon rapid diagnosis and prompt initiation of antifungal agents PCR-RFLP proves to be a rapid and reliable test to identify most of the prevailing species of *Candida*.

References:

1. Safavieh M, Coarsey C, Esiobu N, Memic A, Vyas JM, Shafiee H, et al. Advances in *Candida* detection platforms for clinical and point-of-care applications [Internet]. Vol. 37, Critical Reviews in Biotechnology. 2017. p. 441–58. Available from: http://dx.doi.org/10.3109/07388551.2016.1167667
2. Vijayakumar R, Giri S, Kindo AJ. Molecular Species Identification of *Candida* from Blood Samples of Intensive Care Unit Patients by Polymerase Chain Reaction – Restricted Fragment Length Polymorphism [Internet]. Vol. 4, Journal of Laboratory Physicians. 2012. p. 001–4. Available from: http://dx.doi.org/10.4103/0974-2727.98661
3. Tellapragada C, Eshwara VK, Johar R, Shaw T, Malik N, Bhat PV, et al. Antifungal Susceptibility Patterns,In VitroProduction of Virulence Factors, and Evaluation of Diagnostic Modalities for the Speciation of PathogenicCandidafrom Blood Stream Infections and Vulvovaginal Candidiasis [Internet]. Vol. 2014, Journal of Pathogens. 2014. p. 1–8. Available from: http://dx.doi.org/10.1155/2014/142864
4. Shivaprakasha S, Radhakrishnan K, Karim PMS. Candida spp. other than Candida albicans: a major cause of fungaemia in a tertiary care centre. Indian J Med Microbiol. 2007 Oct;25(4):405–7.
5. Khadka S, Sherchand JB, Pokhrel BM, Parajuli K, Mishra SK, Sharma S, et al. Isolation, speciation and antifungal susceptibility testing of Candida isolates from various clinical specimens at a tertiary care hospital, Nepal [Internet]. Vol. 10, BMC Research Notes. 2017. Available from: http://dx.doi.org/10.1186/s13104-017-2547-3
6. Das I, Nightingale P, Patel M, Jumaa P. Epidemiology, clinical characteristics, and outcome of candidemia: experience in a tertiary referral center in the UK [Internet]. Vol. 15, International Journal of Infectious Diseases. 2011. p. e759–63. Available from: http://dx.doi.org/10.1016/j.ijid.2011.06.006
7. Lone SA, Ahmad A. Candida auris-the growing menace to global health. Mycoses. 2019 Aug; 62(8):620–37.
8. Chakrabarti A, Sood P, Rudramurthy SM, Chen S, Kaur H, Capoor M, et al. Incidence, characteristics and outcome of ICU-acquired candidemia in India [Internet]. Vol. 41, Intensive Care Medicine. 2015. p. 285–95. Available from: http://dx.doi.org/10.1007/s00134-014-3603-2
9. Rudramurthy SM, Chakrabarti A, Paul RA, Sood P, Kaur H, Capoor MR, et al. Candida auris candidaemia in Indian ICUs: analysis of risk factors [Internet]. Vol. 72, Journal of Antimicrobial Chemotherapy. 2017. p. 1794–801. Available from: http://dx.doi.org/10.1093/jac/dkx034
10. Sarma S, Upadhyay S. Current perspective on emergence, diagnosis and drug resistance in Candida auris [Internet]. Vol. 10, Infection and Drug Resistance. 2017. p. 155–65. Available from: http://dx.doi.org/10.2147/idr.s116229
11. Ahmad S, Khan Z, Al-Sweih N, Alfouzan W, Joseph L, Asadzadeh M. Candida kefyr in Kuwait: Prevalence, antifungal drug susceptibility and genotypic heterogeneity. PLoS One. 2020 Oct 27;15(10):e0240426.
12. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of Candidemia in a Tertiary Care Centre of North India: 5-Year Study [Internet]. Vol. 35, Infection. 2007. p. 256–9. Available from: http://dx.doi.org/10.1007/s15010-007-6144-6
13. Giri S, Kindo AJ, Kalyani J. Candidemia in intensive care unit patients: a one year study from a tertiary care center in South India. J Postgrad Med. 2013 Jul;59(3):190–5.

Quiz: Can you identify the fungus?

A -50year-old male patient, farmer by occupation, presented with erythematous plaque over the left forearm of 3 years duration. There was no history of pain, itching, discharge or bleeding from the lesion. Patient was not a diabetic nor on medication for any ailment. Cutaneous examination revealed a well-demarcated, erythematous, indurated, non-tender and crusted plaque measuring 4 × 4 cm. Skin biopsy was performed and subjected to microbiological evaluation which included 10% potassium hydroxide (KOH) mount and culture on Sabouraud dextrose agar (SDA). KOH mount showed ribbon like broad aseptate hyphae. SDA culture at 25°C on day 7 showed cottony, buff coloured growth which filled the tube (Figure A). The lactophenol cotton blue mount from culture in shown in figure B. Please identify the fungus to species level.

Figure A

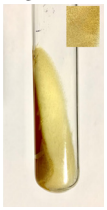
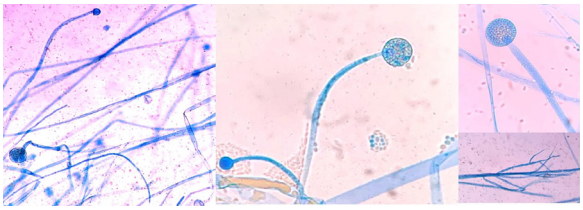


Figure B



Send your answer to Dr Harsimran Kaur at drharsimranpgi@gmail.com

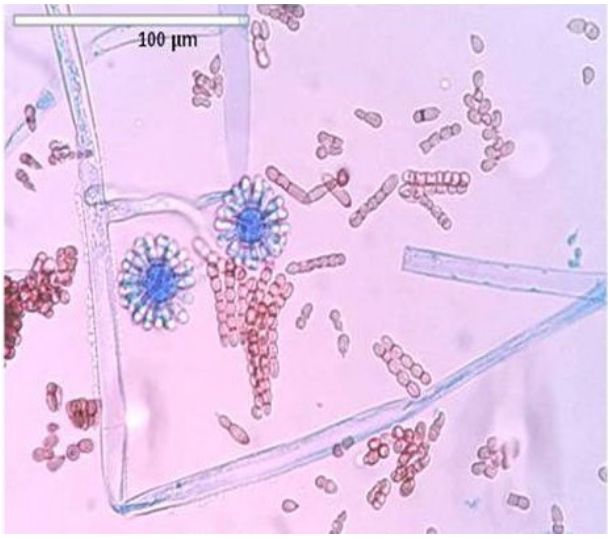
Answer for the last Issue's identify the fungus (ISMM mycoses, Issue 21, Quiz June 2020)

Answer for the last Issue's identify the fungus (ISMM mycoses, Issue 21, Quiz June 2020)

A 44-year-old male patient presented with complaints of painless, diffuse swelling in the lateral aspect of the right thigh for the past four months. He gave history of incision and drainage of the swelling for suspected abscess two months back. He has no history suggestive of immunosuppression or steroid therapy and was seronegative for HIV. Examination of the swelling revealed a hard indurated lesion of 14 x 10 cm with well-defined margins. Within one week of hospitalisation, the patient developed high grade fever with increase in the size of the swelling and tenderness at the local site and multiple discharging sinuses with focal areas of cutaneous necrosis. Local debridement was performed and the tissue subjected to microbiological evaluation which included 10% Potassium hydroxide (KOH) mount and culture on Sabouraud dextrose agar. The culture on SDA shows cottony to fluffy mycelial grayish brown growth which also grew at a temperature of 42°C. Microscopic features included broad aseptate hyphae with erect sporangiophores bearing terminal, globose-to ovoid vesicles surrounded by finger like merosporangia roughly 7.3 ± 2 µm in size. Each merosporangia contained approximately 3-6 large merospores (4 ± 0.5 µm in size) and only a few adventitious rhizoids and was identified as *S. monosporum* . This differs from *S. racemosum* which has longer merosporangia (ranging from 18 to 25 µm in length) with more number of merospores (range: 10 – 25) of smaller size (1.6 to 3 µm) and abundant rhizoids.

Correct identification: *Syncephalastrum monosporum.* Identified to genus level by Dr. Archana Choure, Smt. Kashibai Navale Medical College, Pune and Dr. Manuel Thomas, Unibiosys Biotech Research Labs. Cochin.

Last quiz lactophenol cotton blue mount picture



Results of ISMM Mycology External Quality Assurance Program conducted at PGIMER, Chandigarh

Performance Report of the Participants (23rd Batch, July 2020) Total number of participating laboratories -125

S No.	Sample/ Code	Clinical details			Correct identification	Interpretation	Laboratory (%) given correct results
		Age/Sex	Clinical feature/ Diagnosis	Source of specimen			
1	EQMM-1	40yrs/F	Tinea corporis	Skin scrapings	*Trichophyton rubrum*	Dermatophytosis	92.5%
2	EQMM-2	25yrs/M	Brain abscess	Pus	*Exophiala dermatitidis*	Cerebral phaeohy-phomycosis	74.4%
3	EQMM-3	30yrs/M	Roadside accident, sub-cutaneous gangrene	Skin biopsy	*Rhizopus microsporus*	Cutaneous mucormycosis	70.2%
4	EQMM-4	60yrs/F	Nodules in upper lobe of both lungs	BAL	*Aspergillus fumigatus*	Invasive pulmonary aspergillosis	91.4%
5	EQMM-5*	50yrs/M	Intra abdominal abscess	Drain fluid	*Candida parapsilosis*	Intra-abdominal candidiasis	80.8%

***Results of antifungal susceptibility testing performed for EQMM-5/20 Laboratories participating in AFST –74.3 %**

(EQMM-5) Minimum inhibitory concentration	Fluconazole 0.5mg/L	Voriconazole 0.03mg/L	Itraconazole 0.03mg/L	Posaconazole 0.03mg/L	Amphotericin B 0.25mg/L	Caspofungin 1mg/L	Anidulafungin 1mg/L
Participant results (%)	100%	100%	53.6%	52.1%	97.1%	91.3%	18.8%

13th Annual Workshop (Virtual Workshop) in Basic and Molecular Mycology: a report

Anupma jyoti Kindo

Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Porur, Chennai – 600116, India.

I am glad to inform you that 13th Annual Workshop in Basic and Molecular Mycology" was conducted as a "Virtual workshop"at Sri Ramachandra Medical college and Research Institute by the Department of Microbiology , Mycology division. Two hundred and seventy participants had enrolled for the workshop which included faculty, postgraduate students and technologists in Microbiology from all over India and abroad.

We had reputed faculty as usual: Dr. Arunaloke Chakrabarti, Professor & Head, Department of Microbiology, PGIMER-Chandigarh, Dr. Savitri Sharma, Director, lab. services-LVPEI- Hyderabad, Dr. Madhu, Associate Professor, Deparmtent of Dermatology Madras Medical College- Chennai, Dr. Jayanthi Savio, Professor, St. Johns Medical college-Bangalore, Dr. Rungmei S. K. Marak, Professor, Sanjay Gandhi Postgraduate Institute of Medical Sciences-Lucknow, Dr. Sandhya Sundharam, Professor and Head department of Pathology –SRMC and RI , Chennai.

Conidiogenesis and fungal morphology was shown by Dr. Arunaloke Chakrabarti with the videos and direct demonstration of fungal identification. Clinical manifestations of Dermatophytes were exclusively shown and discussed with Dr. Madhu. Fungal infections of the eye with diagnostics and identification were shown by Dr. Savithri Sharma.

All hands-on sessions were converted to video with voice over. Almost 12 such videos were made. Over 40 cultures were demonstrated with culture characteristics and LPCB including the pathognonmic features.

Histopathology and Mycology correlation was demonstrated by a session on "Path meets Micro". Histopathology slides on various fungal infections was shown by the pathologists.

We had prizes for designing a Mycology lab, for Quiz and Scientific presentation both oral and poster.

The resoponse for this workshop was over whelmimg. If not for the virtual workshop we would have had only 40 participants.

Abstracts (Jun-Dec 2020)

Compiled by Dr Joveeta Joseph

Microbiologist, Jhaveri Microbiology Centre, L V Prasad Eye Institute, Hyderabad

1. Fungal infection in post-renal transplant patient: Single-center experience

Krishan L Gupta, Sahil Bagai, Raja Ramachandran, Vivek Kumar, Manish Rathi, Harbir S Kohli , Ashish Sharma, Arunaloke Chakrabarti

Postgraduate Institute of Medical Education & Research, Chandigarh, India.

Indian J Pathol Microbiol 2020; 63:587-92.

PURPOSE: To study the clinical profile, etiology, risk factors, treatment, and outcome of fungal infections in post-renal transplant recipients.

METHOD: This was a cross-sectional observational retrospective study from January 2014 to June 2017 wherein renal transplant recipients with invasive fungal infection were included and were followed.

RESULTS: Amongst 550 renal transplant recipients, 56 (10.2%) patients developed invasive fungal infection. Mean age of patients was 40.61 ± 10.06 (13-66) years and mean duration of acquiring infection post-transplant was 25.33 ± 23.65 (1-96) months. Male to female ratio was 3:1. Fever was the commonest presentation observed in 89.3% patients. Cough (76.8%), breathlessness (64.3%), sputum (55.3%), hypoxia (50%), and hemoptysis (10.7%) were other common clinical symptoms at presentation. Mean serum creatinine at presentation was 1.70 mg/dl. Most common invasive fungal infection isolated was Mucormycosis 15 (26.7%), followed by Aspergillosis 13 (23.2%), *Pneumocystis jiroveci* 12 (21.4%), Cryptococcus 6 (10.7%), Candida 4 (7.1%), Histoplasmosis 3 (5.3%), Phaeohypomycosis 2 (3.5%), and 5 (8.9%) patients had undetermined fungal etiology. Twenty (35.7%) patients had evidence of dual infection. Use of antithymocyte globulin 27 (48.2%), post-transplant diabetes mellitus 18 (32.1%), Cytomegalovirus (CMV) infection 16 (28.5%), anti-rejection therapy 9 (16%), and Hepatitis C infection 7 (12.5%) were some identified risk factors. Ten (17.8%) patients had graft loss and 12 (21.4%) patients died in the study period.

Conclusions: Invasive fungal infection is a serious threat to renal transplant recipients. Patient and graft survival is significantly affected by fungal infection in developing world.

2. A multicentre observational study on the epidemiology, risk factors, management and outcomes of mucormycosis in India.

A Patel [1], H Kaur [2], I Xess [3], J S Michael [4], J Savio [5], S Rudramurthy[2], R Singh [6], P Shastri [7], P Umabala [8], R Sardana [9], A Kindo [10], M R Capoor [11], S Mohan [12], V Muthu [13], R Agarwal [13], A Chakrabarti[14]

[1] Department of Infectious Diseases, Sterling Hospital, Ahmedabad, India; Department of Internal Medicine, University of South Florida, Tampa, FL, USA.

[2] Department of Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

[3] Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India.

[4] Department of Clinical Microbiology, Christian Medical College, Vellore, India.

[5] St John's Medical College Hospital, Bangalore, India.

[6] Department of Microbiology, JIPMER, Pondicherry, India.

[7] Intensive Care Medicine, Sir Ganga Ram Hospital, New Delhi, India.

[8] Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, India.

[9] Department of Microbiology, Indraprastha Apollo Hospital, New Delhi, India.

[10] Department of Microbiology, Sri Ramachandra Medical College, Chennai, India.

[11] Vardhman Mahaveer Medical College and Safdarjang Hospital, New Delhi, India.

[12] Department of Microbiology, Christian Medical College, Ludhiana, India.

[13] Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

[14] Department of Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India. Electronic address: arunaloke@hotmail.com.

Clin Microbiol Infect. 2020 Jul;26(7):944.

PURPOSE: To describe the epidemiology, management and outcome of individuals with mucormycosis; and to evaluate the risk factors associated with mortality.

METHODS: We conducted a prospective observational study involving consecutive individuals with proven mucormycosis across 12 centres from India. The demographic profile, microbiology, predisposing factors, management and 90-day mortality were recorded; risk factors for mortality were analysed.

RESULTS: We included 465 patients. Rhino-orbital mucormycosis was the most common (315/465, 67.7%) presentation followed by pulmonary (62/465, 13.3%), cutaneous (49/465, 10.5%), and others. The predisposing factors included diabetes mellitus (342/465, 73.5%), malignancy (42/465, 9.0%), transplant (36/465, 7.7%), and others. Rhizopus species (231/290, 79.7%) were the most common followed by Apophysomyces variabilis (23/290, 7.9%), and several rare Mucorales. Surgical treatment was performed in 62.2% (289/465) of the participants. Amphotericin B was the primary therapy in 81.9% (381/465), and posaconazole was used as combination therapy in 53 (11.4%) individuals. Antifungal therapy was inappropriate in 7.6% (30/394) of the individuals. The 90-day mortality rate was 52% (242/465). On multivariate analysis, disseminated and rhino-orbital (with cerebral extension) mucormycosis, shorter duration of symptoms, shorter duration of antifungal therapy, and treatment with amphotericin B deoxycholate (versus liposomal) were independent risk factors of mortality. A combined medical and surgical management was associated with a better survival.

Conclusions: Diabetes mellitus was the dominant predisposing factor in all forms of mucormycosis. Combined surgical and medical management was associated with better outcomes. Several gaps surfaced in the management of mucormycosis. The rarer Mucorales identified in the study warrant further evaluation.

3. *Aspergillus flavus* necrotising scleritis following pars plana vitrectomy

Abhilasha Baharani[1], Ashok Kumar Reddy[2], Raja Rami Reddy P[3]

[1] Uvea, Neoretina Eyecare Institute, Hyderabad, India.

[2] Microbiology, GHR Micro Diagnostics, Hyderabad, India.

[3] Retina, Neoretina Eyecare Institute, Hyderabad, India.

Ocul Immunol Inflamm. 2020 Jul 3;28(5):772-774.

PURPOSE: To report a case of fungal necrotising scleritis following pars plana vitrectomy.

RESULTS: A 65-year-old lady underwent phacoemlsificication with posterior capsular rupture and posteriorly dislocated lens in her left eye. On the same day she underwent 20 gauge pars plana vitrectomy and phacofragmentation. Postoperative period was uneventful for up to 6 weeks when she developed necrotising anterior scleritis with suppurative nodules. Scraping from the suppuration confirmed the presence of *Aspergillus flavus*. She was treated with topical Voriconazole and oral Itraconazole.

CONCLUSIONS: We describe the first case of fungal necrotising scleritis without intraocular inflammation following pars plana vitrectomy (PubMed Search). Infection should be kept in the differential diagnosis of post-operative necrotising scleritis even in the absence of risk factors like hypopyon or diabetes. Early recognition improves final outcome. Medical therapy should be continued even after presumed cure to take care of residual fungi and prevent recurrences.

4. Amphotericin B loaded ethyl cellulose nanoparticles with magnified oral bioavailability for safe and effective treatment of fungal infection

Kirandeep Kaur[1], Parveen Kumar[2], Preeti Kush[3]

[1] Department of Pharmaceutics, Chandigarh College of Pharmacy, Mohali, Punjab, India.

[2] Nanotechnology Division (H-1), CSIR-Central Scientific Instruments Organization, Chandigarh, 160030, India.

[3] Department of Pharmaceutics, Chandigarh College of Pharmacy, Mohali, Punjab, India.

Biomed Pharmacother. 2020 Aug;128:110297.

PURPOSE: Amphotericin B is a gold standard drug used in various fungal and parasitic infection treatment. Most of the marketed formulations are administered intravenously, but show dose-dependent adverse effects i.e., nephrotoxicity and hemolysis. Oral route eliminates the toxic concern but exhibits poor bioavailability. Therefore, ethylcellulose nanoparticles (EC-NPs) have been used for magnified oral delivery of AmB, where EC provides gastrointestinal stability.

Methods: These nanoparticles were synthesized by high-pressure emulsification solvent evaporation (HPESE) method and evaluated for in vitro and in vivo studies. This method yields small, monodisperse AmB-EC-NPs along with smooth surface morphology and improved encapsulation efficiency.

Results: The developed formulation showed a sustained release pattern following Higuchi diffusion kinetics along with gastric and storage stability. Aggregation study revealed that AmB was present in its monomeric form inside the biocompatible EC matrix. The antifungal result demonstrated that the MIC of AmB-EC-NPs was reduced ~1/3rd than AmB and Fungizone® at 24 h whereas it was observed ~1/8th at 48 h. in vivo pharmacokinetic analysis demonstrated 1.3-fold higher AUC than Fungizone® even at a 4.5-time lesser dose via the oral route and a ~15-fold rise in the bioavailability in contrast to the native AmB. The haemolytic study revealed that the developed formulation.5. Molecular identification of pathogenic fungi in formalin-fixed and paraffin-embedded tissues.

Joseph Jillwin[1,2], Shivaprakash M Rudramurthy[2], Shreya Singh[2], Amanjit Bal[3], Ashim Das[3], Bishan Radotra[3], Hariprasath Prakash[2], Manpreet Dhaliwal[2], Harsimran Kaur [2], Anup K Ghosh[2], Arunaloke Chakrabarti[2]

[1] Present address: Lecturer of Microbiology, Xavier University School of Medicine, Oranjestad, Aruba.

[2] Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

[3] Department of Histopathology, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

J Med Microbiol. 2020 Nov 30. Online ahead of print

PURPOSE: Histopathological examination (HPE) of tissue helps in the diagnosis of invasive fungal infections (IFIs) but cannot identify the fungus to the genus/species level Gap Statement Available protocols for the molecular identification of fungi from formalin-fixed and paraffin-embedded (FFPE) tissues have limitations in terms of extraction and target selection, and standardisation. Development of sequence-based fungal identification protocol after extraction of DNA from formalin-fixed and paraffin-embedded (FFPE) tissues.

METHODS: A total of 63 FFPE tissues from histopathology proven IFI cases were used to standardize the DNA extraction (commercial QIAamp kit-based extraction and conventional phenol-chloroform-isoamyl alcohol [PCI] method) and sequence-based fungal identification protocols. The PCR targeted different ribosomal DNA (rDNA) regions including complete internal transcribed spacer (ITS1-5.8S-ITS2), separate ITS1 and ITS2, 18S and D1/D2 of 28S regions. Semi-nested PCR targeting Mucorales-specific 18S rDNA region was performed in tissues having aseptate hyphae. The optimized ITS1-PCR protocol was evaluated in 119 FFPE tissues containing septate hyphae or yeast, and Mucorales-specific semi-nested PCR in 126 FFPE tissues containing aseptate hyphae.

RESULTS: The DNA yield by conventional PCI method was significantly higher (P<0.0001) than commercial kit, though the quality of DNA was similar by both protocols. The test accuracy was best while using ITS1 (61.9 %) as the target compared to 7.9, 29.9 and 22.2 % on targeting ITS1-5.8S-ITS2, ITS2, the D1/D2 region of 28S, respectively. The test accuracies of ITS1-PCR in tissues containing septate hyphae, aseptate hyphae and yeasts were 75.5, 18.7 and 100 %, respectively. The amplification (targeting ITS1 region) improved by increasing the thickness of tissue section (up to 50 µm) used for DNA extraction. ITS1-PCR protocol could amplify fungal DNA in 76 (63.8 %) tissues and Mucorales-specific semi-nested PCR in 86 (68.3 %) tissues.

CONCLUSIONS: Conventional PCI-based DNA extraction from thick tissue (50 µm) may be used until optimal commercial fungal DNA extraction kit is developed. Subsequent ITS1-PCR for septate fungi and yeast, and semi-nested PCR targeting 18S rDNA for Mucorales are recommended to identify the fungus in FFPE tissues.

6. Mechanistic insights into *Candida* biofilm eradication potential of eucalyptol

P Gupta, V Pruthi, K M Poluri

Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee, Uttarakhand, India.

J Appl Microbiol. 2020 Nov 23. doi: 10.1111/jam.14940. Online ahead of print.

PURPOSE: *Candida*-associated fungal infections are prevalent in hospitalized and immune-compromised patients. Their biofilm architecture and high rate of antifungal resistance make treatment challenging. Eucalyptol (EPTL), a monoterpene majorly present in the essential oil of eucalyptus is well known for curing respiratory infections. Hence, the present study investigated the anti-biofilm efficacy of EPTL against the laboratory strains and clinical isolates of *Candida* to delineate its mode of action.

METHODS: The effect of EPTL on the viability, biofilm formation, and mature biofilm of Candida strains was studied. Furthermore, its effect on cell cycle arrest, mitochondrial membrane potential (MMP), ROS generation, germ tube formation, ergosterol content and transcriptional expression of selected genes was also investigated.

RESULTS: EPTL exhibited anti-biofilm activity against mature and developing biofilm of *Candida albicans* and *Candida glabrata* along with their clinical isolates. The biochemical components and enzyme activity were differentially modulated in EPTL-treated biofilm extracellular matrix. EPTL generated ROS and arrested cell cycle at the G1 /S phase in both the species, while altered MMP was recorded in *C. glabrata*. Transcriptional analysis evidenced for differential gene expression of selected ABC transporters, secreted hydrolytic enzymes, and cell wall biogenesis in *C. albicans/C. glabrata* upon treating with EPTL.

CONCLUSIONS: The current data on anti-biofilm activity of EPTL establish its candidacy for drug development or as an adjuvant with existing antifungal formulations.

7. Utility of nested polymerase chain reaction for fungus in detecting clinically suspected patients of invasive fungal infections and its clinical correlation and comparison with fungal culture

Vibhu Ranjan Khare, Munesh Kumar Gupta, Nilesh Kumar, Ranjan Bhattnagar , Ragini Tilak, Kailash Kumar, Chandan Kumar, Anurag Rana

Department of General Medicine and Microbiology, IMS, BHU, Varanasi, Uttar Pradesh, India.

J Family Med Prim Care. 2020 Sep 30;9(9):4992-4997.

PURPOSE: The aim and objective of this study is to detect invasive fungal infections (IFIs) early and with more sensitivity by the nested polymerase chain reaction (PCR) for fungus as compared to fungal culture in clinically suspected patients and also explore its correlation in reference to age, duration of symptoms, immunocompromised status, and other risk factors predisposing to IFIs.

METHODS: In this cross-sectional study, 50 suspected patients admitted in medical acute care unit/intensive care unit (ACU/ICU) of Sir Sunderlal Hospital, Banaras Hindu University, Varanasi, India, comprised the study. All cases were selected based on the predefined inclusion and exclusion criteria. A detailed history, clinical examination, and all required investigations were done in all suspected patients. Blood samples were taken for nested-PCR for fungus and culture. Nested PCR was performed on extracted DNA form samples collected from all participants under the study.

RESULTS: Our study comprised of 50 suspected immunocompromised patients of IFIs. Among the participants under the study, the most common risk factor was diabetes mellitus (28% cases). Nearly two-thirds (60%) of the cases were 50 years or more. Around 70% of the cases had a history of illness more than 2 weeks. Nested PCR for fungus came out to be positive in 21/50 patients (42%); however, fungal culture was positive in none. Among the admitted patient in ACU/ICU, 75% were neutropenic.

CONCLUSIONS: IFIs are more common in immunocompromised individuals, patients with comorbidities, long history of symptoms, and elderly population. Nested PCR for fungus has a high sensitivity (as compared to the fungal culture), and also they are rapid in giving the results. Thus, nested PCR for fungus can be used in a cost-effective manner for the early and reliable diagnosis of clinically suspected IFIs.

8. Inhibitory Effect of Morin Against *Candida albicans* Pathogenicity and Virulence Factor Production: An in vitro and in vivo Approaches

Gurusamy Abirami1, Rajaiah Alexpandi[1], Ravindran Durgadevi[1], Arunachalam Kannappan[1,2], Arumugam Veera Ravi[1]

[1] Department of Biotechnology, School of Biological Sciences, Alagappa University, Karaikudi, India.

[2] Department of Food Science and Technology, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China.

Front Microbiol. 2020 Oct 23;11:561298.

PURPOSE: *Candida albicans* is considered an exclusive etiologic agent of candidiasis, a very common fungal infection in human. The expression of virulence factors contributes highly to the pathogenicity of *C. albicans*. These factors include biofilm formation, yeast-to-hyphal transition, adhesins, aspartyl proteases, and phospholipases secretion. Moreover, resistance development is a critical issue for the therapeutic failure of antifungal agents against systemic candidiasis.

METHODS: To circumvent resistance development, the present study investigated the virulence targeted therapeutic activity of the phyto-bioactive compound morin against C. albicans. Morin is a natural compound commonly found in medicinal plants and widely used in the pharmaceutical and cosmetic products/industries. The present study explicated the significant inhibitory potential of morin against biofilm formation and other virulence factors' production, such as yeast-hyphal formation, phospholipase, and exopolymeric substances, in C. albicans.

RESULTS: qPCR analysis confirmed the downregulation of biofilm and virlence-related genes in C. albicans upon morin treatment, which is in correspondence with the *in vitro* bioassays. Further, the docking analysis revealed that morin shows strong affinity with Hwp-1 protein, which regulates the expression of biofilm and hyphal formation in *C. albicans* and, thereby, abolishes fungal pathogenicity. Moreover, the anti-infective potential of morin against *C. albicans*-associated systemic candidiasis is confirmed through an in vivo approach using biomedical model organism zebrafish (Danio rerio). The outcomes of the in vivo study demonstrate that the morin treatment effectively rescues animals from *C. albicans* infections and extends their survival rate by inhibiting the internal colonization of *C. albicans*. Histopathology analysis revealed extensive candidiasis-related pathognomonic changes in the gills, intestine, and kidney of animals infected with *C. albicans*, while no extensive abnormalities were observed in morin-treated animals.

CONCLUSIONS: The results evidenced that morin has the ability to protect against the pathognomonic effect and histopathological lesions caused by *C. albicans* infection in zebrafish. Thus, the present study suggests that the utilization of morin could act as a potent therapeutic medication for *C. albicans* instigated candidiasis.

9. Interactions between *Candida albicans* and *Enterococcus faecalis* in an Organotypic Oral Epithelial Model

Akshaya Lakshmi Krishnamoorthy[1], [2], Alex A Lemus[3], Adline Princy Solomon[2], Alex M Valm[3,4] , Prasanna Neelakantan[1]

[1] Faculty of Dentistry, The University of Hong Kong, Pok Fu Lam, Hong Kong.

[2] Quorum Sensing Laboratory, Center of Research in Infectious Diseases, School of Chemical and Biotechnology, SASTRA Deemed to be University, Thanjavur 613401, India.

[3] Department of Biological Sciences, University at Albany, State University of New York, Albany, NY 12222, USA.

[4] The RNA Institute, University at Albany, State University of New York, Albany, NY 12222, USA.

Microorganisms. 2020 Nov 11;8(11):1771.

PURPOSE: *Candida albicans* as an opportunistic pathogen exploits the host immune system and causes a variety of life-threatening infections. The polymorphic nature of this fungus gives it tremendous advantage to breach mucosal barriers and cause oral and disseminated infections. Similar to *C. albicans*, *Enterococcus faecalis* is a major opportunistic pathogen, which is of critical concern in immunocompromised patients. There is increasing evidence that *E. faecalis* co-exists with *C. albicans* in the human body in disease samples. While the interactive profiles between these two organisms have been studied on abiotic substrates and mouse models, studies on their interactions on human oral mucosal surfaces are non-existent.

METHODS: Here, for the first time, we comprehensively characterized the interactive profiles between laboratory and clinical isolates of *C. albicans* (SC5314 and BF1) and *E. faecalis* (OG1RF and P52S) on an organotypic oral mucosal model.

RESULTS: Our results demonstrated that the dual species biofilms resulted in profound surface erosion and significantly increased microbial invasion into mucosal compartments, compared to either species alone. Notably, several genes of *C. albicans* involved in tissue adhesion, hyphal formation, fungal invasion, and biofilm formation were significantly upregulated in the presence of *E. faecalis*. By contrast, E. faecalis genes involved in quorum sensing, biofilm formation, virulence, and mammalian cell invasion were downregulated.

CONCLUSION: This study highlights the synergistic cross-kingdom interactions between *E. faecalis* and *C. albicans* in mucosal tissue invasion.

10. Fungal osteomyelitis and soft tissue infections: Simple solutions to uncommon scenarios

Ravi Chavan, Aditya Menon, Rajeev Soman, Camilla Rodrigues, Anjali Shetty, Ravi Bhadiyadra, Vikas M Agashe

P.D. Hinduja Hospital and Medical Research Centre, Mahim, Mumbai, India.

J Infect Dev Ctries. 2020 Sep 30;14(9):1033-1039.

Purpose: Fungal osteoarticular/soft tissue infections (FOaSI) are an uncommon entity with protracted course due to variability in clinical picture, slow progression; resulting in misdiagnosis with empirical therapy. Recent studies have shown an alarming emergence of FOaSI in immunocompetent individuals with high mortality rates. This study recommends a protocol for managing these complex and confusing scenarios.

Methods: We have retrospectively analysed patients with FOaSI between January 2014 and December 2016, with a minimum 12 months follow up.

RESULTS: 8 cases (6 male, 2 female) with a mean age of 42.88 years (26-53) presented to us 45 days (3-365) after initial symptoms. They underwent mean 3 procedures before being diagnosed with a fungal infection. Deep tissue cultures grew 9 fungi and 6 bacteria, commonest fungus being Candida sp (n = 4), treated with appropriate antifungals and antibiotics. Infection remission was achieved in 7/8 (87.5%) cases at 27.1 months (19-45) follow-up with 1 mortality. Excellent functional results as per our criteria were seen in 5 cases (62.5%) with 1 talus excision, 1 ray amputation and 1 mortality.

CONCLUSIONS: This study highlights the significance of implementing a simple rule such as obtaining fungal cultures in every case of bone and soft tissue infections. Standardisation of treatment may not be the ideal solution, since different fungi have different growth patterns and invasiveness. A simple protocol of customising the medico- surgical treatment with an open ended discussion between the surgeons, microbiologists, pathologists and infectious disease specialists forms the cornerstone to success.

11. Colloidal lipid nanodispersion enriched hydrogel of antifungal agent for management of fungal infections: Comparative *in-vitro*, *ex-vivo* and *in-vivo* evaluation for oral and topical application

Narendar Dudhipala1, Ahmed Adel Ali Youssef2, Nagaraj Banala3

[1]Department of Pharmaceutics, Vaagdevi College of Pharmacy, Warangal, 50 6005, Telangana State, India. Electronic address: narphmreddy@gmail.com.

[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, Kafrelsheikh University, Kafrelsheikh, 33516, Egypt.

[3]Department of Nanotechnology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana State, India.

Chem Phys Lipids. 2020 Nov;233:104981.

PURPOSE: Ketoconazole (KZ) is broad spectrum antifungal drug, used for the treatment of fungal infections. KZ's clinical topical use has been associated with some adverse effects in healthy adults particularly local reactions, such as stinging, severe irritation, and pruritus. However, bioavailability of KZ after oral administration is low from tablets due to its low aqueous solubility.

METHODS: The objective of this investigation was development and characterization of KZ-containing solid lipid nanoparticles (KZ-SLNs) and SLN-containing hydrogel (KZ-SLN-H) for oral and topical delivery of KZ. KZ-SLNs were prepared using homogenization-sonication method. Optimal KZ-SLN formulation was selected based on physicochemical and in-vitro release studies. Optimized KZ-SLN converted to KZ-SLN hydrogel (KZ-SLN-H) using gelling polymers and optimized with rheological and in-vitro studies. Further, optimized KZ-SLN and KZ-SLN-H formulations evaluated for crystallinity, morphology, stability, ex-vivo and in-vivo pharmacokinetic (PK) studies in rats, comparison with KZ suspension (KZ-S) and KZ-S hydrogel (KZ-SH).

RESULTS: Optimized KZ-SLN formulation showed desirable characters. KZ-SLN and KZ-SLN-H formulations exhibited spherical shape, converted to amorphous, sustained release behaviour and enhanced permeability ($p < 0.05$). Moreover, both formulations were stable for three months at 4 °C and 25 °C. PK studies revealed 1.9 and 1.5-folds, 3.5 and 2.8-folds enhancement of bioavailability of optimized KZ-SLN and KZ-SLN-H formulations ($p < 0.05$) compared with KZ-S and KZ-SH formulations, respectively.

Conclusions: SLN and SLN-H formulations could be considered as an efficient delivery vehicles for KZ through oral and topical administration for better control over topical and systemic fungal infections.

12. Antimicrobial peptides in human corneal tissue of patients with fungal keratitis

Imran Mohammed [1], Debasmita Mohanty [2], Dalia G Said[1,3], Manas Ranjan Barik[4], Mamatha M Reddy[4], Ahmed Alsaadi[5], Sujata Das[6], Harminder Singh Dua [1,3], Ruchi Mittal[2]

[1]Academic Ophthalmology, Division of Clinical Neuroscience, School of Medicine, University of Nottingham, Nottingham United Kingdom.

[2]Kanupriya Dalmia Ophthalmic Pathology Laboratory, L V Prasad Eye Institute, MTC Campus, Bhubaneswar, India.

[3]Ophthalmology Department, Nottingham University Hospitals, Queens Medical Centre, Nottingham United Kingdom.

[4]Ocular Microbiology Service, L V Prasad Eye Institute, MTC Campus, Bhubaneswar, India.

[5]Ophthalmology Department, Zayed Military Hospital, Abu Dhabi, United Arab Emirates.

[6]Cornea and Anterior Segment Service, L V Prasad Eye Institute, MTC Campus, Bhubaneswar, India.

Br J Ophthalmol. 2020 Aug 27;bjophthalmol-2020-316329.

PURPOSE: Fungal keratitis (FK) is the leading cause of unilateral blindness in the developing world. Antimicrobial peptides (AMPs) have been shown to play an important role on human ocular surface (OS) during bacterial, viral and protozoan infections. In this study, our aim was to profile a spectrum of AMPs in corneal tissue from patients with FK during the active phase of infection and after healing.

METHODS: OS samples were collected from patients at presentation by impression cytology and scraping. Corneal button specimens were collected from patients undergoing therapeutic penetrating keratoplasty for management of severe FK or healed keratitis. Gene expression of human beta-defensin (HBD)-1, -2, -3 and -9, S100A7, and LL-37 was determined by quantitative real-time PCR.

RESULTS: Messenger RNA expression (mRNA) for all AMPs was shown to be significantly upregulated in FK samples. The levels of HBD-1 and -2 mRNA were found to be elevated in 18/20 FK samples. Whereas mRNA for HBD-3 and S100A7 was upregulated in 11/20 and HBD9 was increased in 15/20 FK samples. LL-37 mRNA showed moderate upregulation in 7/20 FK samples compared with controls. In healed scar samples, mRNA of all AMPs was found to be low and matching the levels in controls.

CONCLUSIONS: AMP expression is a consistent feature of FK, but not all AMPs are equally expressed. HBD-1 and -2 are most consistently expressed and LL-37 the least, suggesting some specificity of AMP expression related to FK. These results will help to identify HBD sequence templates for designing FK-specific peptides to test for therapeutic potential.

14. Role of recombinant *Aspergillus fumigatus* antigens in diagnosing *Aspergillus* sensitisation among asthmatics

Valliappan Muthu[1], Pawan Singh[1], Hansraj Choudhary [2], Sahajal Dhooria[1], Inderpaul Singh Sehgal[1], Kuruswamy Thurai Prasad[1], Ashutosh Nath Aggarwal[1], Mandeep Garg[3], Arunaloke Chakrabarti[2], Ritesh Agarwal[1]

[1] Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

[2] Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

[3] Department of Radiodiagnosis and Imaging, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

Mycoses. 2020 Sep;63(9):928-936.

PURPOSE: To evaluate the utility of recombinant *A. fumigatus* (rAsp) antigens in detecting ASA and their role in differentiating true from cross-sensitisation.

METHODS: We performed IgE against rAsp (f 1, f 2, f 3, f 4 and f 6), cAsp and other fungal (Alternaria, Candida, Cladosporium, Malassezia and Trichophyton) antigens in subjects with A fumigatus-unsensitised asthma (Af-UA [n = 51]), ASA (n = 71) and ABPA (n = 123). The diagnoses were made using cAsp-IgE and compared using rAsp-IgE. Subjects with elevated cAsp-IgE, but negative rAsp f 1 and f 2, were presumed to lack true A fumigatus sensitisation.

RESULTS: The prevalence of any rAsp antigen positivity (cut-off, 0.35 kUA/L) varied from 2%-22%, 32%-73% and 84%-98% for Af-UA, ASA and ABPA, respectively. The prevalence of sensitisation to other fungi ranged from 29%-65%, 59%-85% and 87%-95%, respectively, among subjects with Af-UA, ASA and ABPA. Nineteen subjects of ASA and one subject with ABPA were positive with cAsp-IgE but negative for rAsp f 1 and f 2 and were also cross-sensitised to at least one of the other fungi. Five subjects of Af-UA (cAsp-IgE negative) were rAsp f 1 or f 2 positive.

CONCLUSIONS: Crude *Aspergillus* antigens may misclassify Aspergillus sensitisation among asthmatics. IgE against rAsp antigens (f 1 and f 2) potentially detect true Aspergillus sensitisation and could be used for this purpose.

ISMM Mycoses Indian Society of Medical Mycologists Newsletter

Editor: Dr. Savitri Sharma, Jhaveri Microbiology Center, L V Prasad Eye Institute, Hyderabad - 500034, Telangana, India. Phone: (O) +91 40 68102504, Fax: +91 40 68102535, E-mail: savitri@lvpei.org

Editorial Board: Dr. Malini R Capoor, Dr. P. Umabala, Dr. Lalitha Prajna, Dr. Harsimran Kaur

Editorial Assistants: Dr. Ruchi Mittal, Dr. Mamatha Ballal, Dr. Joveeta Joseph, Dr. Shukla Das, Dr. Anuradha Sharma