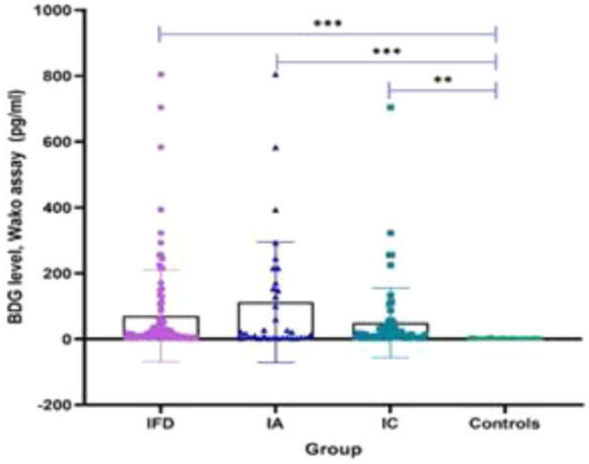
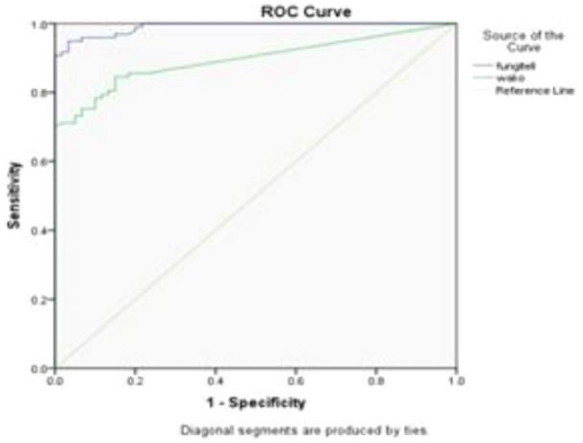


Figure 1. Comparative BDG levels observed in various patient groups using the Wako BDG estimation assay



Receiver operating characteristics (ROC) curve showing the sensitivity and false positive rates (1- specificity) in detecting IFI (proven/ probable) using Fungitell BDG assay (blue) and Wako assay (green)

(70.79 vs. 3.03, *P*-value: .0002), IA vs. controls (112.3 vs. 3.034, *P*-value: < .0001), and IC vs. controls (49.4 vs. 3.034, *P*-value: .0009). A good performance with an AUC of 0.990 for Fungitell and 0.895 for Wako assay was seen. The Youden's index on using the Wako assay at BDG cutoff of 11 pg/mL, 5 pg/ml, and 4 pg/ml were 0.598, 0.677, and 0.664 respectively. Based on this the cut-off of 5 pg/ml was selected as the optimum cutoff for the Wako BDG assay with a sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value and negative predictive value of 79.38%, 85%, 5.43, 0.22, 88.8%, and 73.9% respectively, for diagnosis of IFD. The percentage agreement between Wako and Fungitell assay was 84.7% with a Cohen's k score of 0.691. The mean time to positive test result (i.e., > 5 pg/ml) was 37 min and the negative result was 87 min using the Wako assay compared with 120 min for the Fungitell test. However, an additional 15 min pre-treatment step was required in the Wako assay.

Conclusion: We observed that the performance of Wako assay is comparable to Fungitell assay. Results favored a lower cutoff value of 5 pg/ml compared with the kit specified cutoff of 11 pg/ml. Additionally, Wako assay gives positive results faster than Fungitell by allowing real-time observation. Further multi-centric studies on larger populations are required to establish the equivalence of these assays to guide clinicians in the diagnosis of IFDs.

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Clinical spectrum and molecular characterization of *Rhytidhysteron* spp. (now Gloniopsis) from India

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

Objective: *Rhytidhysteron* spp. are emerging dematiaceous fungi implicated in subcutaneous infections in immunocompromised as well as immunocompetent individuals. Diagnosis of *Rhytidhysteron* spp is delayed owing to non-sporulation and misidentification. The present study was conducted to characterize *Rhytidhysteron* spp. isolated from India by molecular techniques.

Method: We retrieved a total of eight isolates of *Rhytidhysteron* spp from our National Culture Collection for Pathogenic Fungi (NCCPF) that were received from various centers across India. Colony morphology on Sabouraud's dextrose agar (SDA) and potato dextrose agar (PDA) was recorded. Molecular identification was performed by sequencing internal transcribed spacer (ITS) and 28 s region of rDNA. Phylogenetic tree was constructed using the Neighbor-Joining method along with sequences of standard strains retrieved from the NCBI database.

Results: *Rhytidhysteron* spp was confirmed by sequencing from cutaneous specimens of eight cases. Six patients were immunocompetent while two were post-renal transplant recipients. Phenotypic features on SDA and PDA revealed growth of greyish-black mycelia on the obverse with black pigmentation on reverse after 7 days of incubation. On microscopic examination of lactophenol cotton blue mount, only dematiaceous septate hyphae without any spores were noted. On phylogenetic analysis of 28 s region, four of our isolates were closely clustered with *Gloniopsis calami* (Fig.1b), whereas two isolates with *G. pneumonia* and one each with *G. percutanea*, and *Gloniopsis* spp. (Fig.1b). On ITS phylogeny, the same four isolates closely clustered with *G. calami* (Fig.1a) as in 28 s region, one each isolate clustered with *G. pneumonia*, and *G. percutanea* while two isolates formed a separate clade with *Rhytidhysteron rufulum* (Fig.1a).

Conclusion: This fungus is often difficult to identify due to lack of sporulation making morphological identification challenging. Therefore, molecular sequencing is a must for its identification. However, identification using the sequence of ITS and 28 s rDNA does not clearly correlate. Hence to confirm the identity additional genes needs to be sequenced and analyzed.

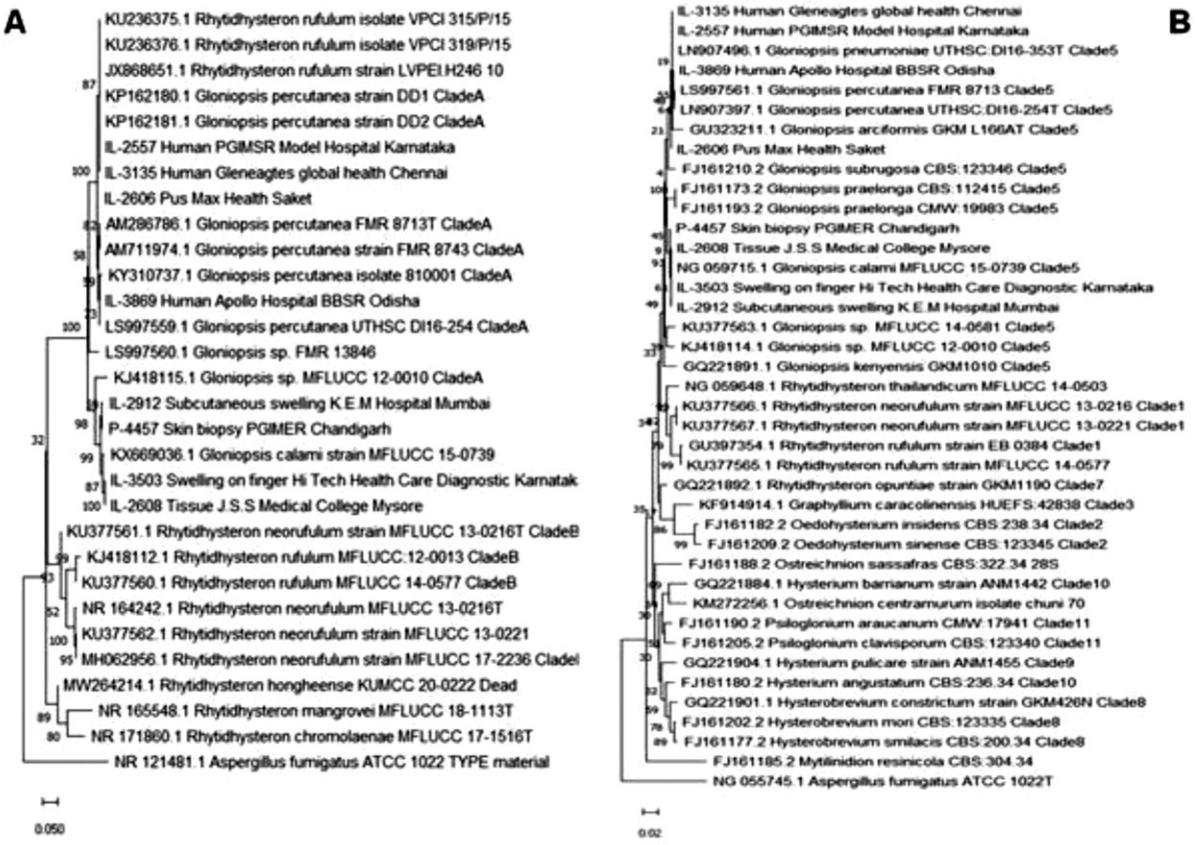

Figure 1. Phylogenetic tree constructed based on ITS (A) and LSU (B) sequences of *Rhytidhysteron* species

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Evaluation of diagnostic potential of recombinant secretory aspartyl proteinase 2 (Sap2) protein from *Candida parapsilosis* for use in systemic candidiasis

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Objectives: Systemic candidiasis is the fourth most common bloodstream infection in ICU patients worldwide. Along with *Candida albicans*, infections caused by non-*albicans Candida* (NAC) species are increasingly becoming more prevalent globally along with the emergence of drug resistance. The diagnosis of systemic candidiasis is difficult due to the absence of significant clinical symptoms in patients. Since conventional diagnostics methods for candidiasis show less sensitivity and specificity, novel immunodiagnostic techniques are needed for early diagnosis. We investigated the diagnostic potential of recombinant secreted aspartyl proteinase 2 (rSap2) from *C. parapsilosis* for the detection of *Candida* infection.

Methods: Genomic DNA was isolated from *C. parapsilosis*, followed by PCR amplification of Sap2 gene using designed gene-specific primers. Sap2 protein expression and purification was performed using Ni-NTA affinity chromatography under denaturing conditions. The denatured protein was subsequently refolded using a multi-step dialysis procedure. CD and FTIR studies were performed to confirm refolding. Mice were immunized with rSap2 protein and serum ELISA assays were performed for testing immunogenicity. Immunoblotting assays and human serum ELISAs were performed using whole-cell *Candida* and rSap2 protein in proven systemic candidiasis patient serum and controls, recruited at PGIMER, Chandigarh.

Results: Sap2 protein from *C. parapsilosis* was successfully cloned and expressed using an E. coli-based prokaryotic expression system. Protein refolding was performed *in vitro* using step-wise dialysis. Structural analysis by CD and FTIR spectroscopy revealed the refolded protein to be in its near native conformation. Immunogenicity analysis demonstrated the rSap2 protein to be highly immunogenic as evident from significantly high titers of Sap2-specific antibodies in antigen immunized BALb/c mice, compared to sham-immunized controls. The diagnostic potential of rSap2 protein was evaluated using immunoblotting and ELISA assays using serum from proven systemic candidiasis patients and controls. Our immunoblotting results demonstrate that the recombinant Sap2 protein was recognized as a single band of approximately 41 kDa by systemic candidiasis patient serum samples and no cross reactivity was observed in healthy controls. On evaluating the diagnostic potential of the rSap2 antigen using an ELISA-based approach, our results show that anti-Sap2 Ig, IgG and IgM antibodies could be detected in the sera of proven candidiasis patients. Of note, the differences in Sap2 antibody titers observed amongst patients and controls were similar to the serological response observed when heat-killed whole-cell *Candida* was used as a coating antigen.

Conclusion: In summary, the rSap2 protein from *C. parapsilosis* has the potential to be used in the diagnosis of systemic candidiasis, providing a rapid, convenient, accurate, and cost-effective strategy. Our results indicate that the rSap2 protein from *C. parapsilosis* can be used to detect and diagnose systemic candidiasis infection in human patients and can be used as an alternative/replacement of whole-cell *Candida*-based ELISA procedures, which are currently in use.

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Clinical utility of semi-nested conventional PCR for diagnosis of mucormycosis in fresh clinical samples

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Objectives: We aim to assess the clinical utility of the semi-nested conventional PCR in smear positive, culture-negative clinical samples for diagnosis of mucormycosis.

Methods: This prospective study was conducted for a period of 3 months (April-June 2021). A total of 218 clinical samples were included from patients in whom smear was positive for aseptate hyphae, but the culture failed to grow within 2 days of incubation or smear was negative but had high suspicion of mucormycosis. Molecular diagnosis was attempted using semi-nested PCR with Mucorales-specific primers targeting 18S region, described previously by Bialek et al.[1]. Phenol-chloroform based manual DNA extraction protocol was optimized in the mycology laboratory of the department of medical microbiology, PGIMER, Chandigarh. This method was applicable to different types of clinical samples to yield good quality DNA with minimal chances of extraneous contamination (Fig. 1). Amplified PCR products were further sequenced to identify the causative species (Fig. 2).

Results: Among 218 patients with suspected mucormycosis included in this study, the majority were rhino-orbito-cerebral mucormycosis (ROCM), (77.7%, $n = 169$), followed by pulmonary mucormycosis (19.2%, $n = 42$), cutaneous (0.02%, $n = 4$), and gastro-intestinal (GI) mucormycosis (0.01%, $n = 3$). In 24 samples, the presence of both septate and aseptate hyphae was seen under microscopic examination raising the possibility of mixed infection. On microscopic examination, 90.3% samples (197/218) had aseptate hyphae while the remaining samples were smear-negative but had strong clinical suspicion of mucormycosis. The molecular technique was able to identify causative agent in 154 culture-negative samples (81.9%, 154/188) and 52.4% (11/21) in smear-negative cases. Among 218 patients, only 20 samples show delayed growth of Mucorales, and on comparison with molecular results 100% concordance was observed. However, 10 patients with strong clinical suspicion for mucormycosis were negative by both conventional and molecular methods. The low culture positivity necessities the molecular diagnosis based on in-house semi-nested PCR using above-mentioned primers followed by Sanger sequencing. In the case of 24 mixed infections with aseptate-septate hyphae, Mucorales-specific PCR correctly identified 23/24 (95.8%) Mucorales as a causative agent. The overall turn-around-time from the sample receiving to diagnosis was <48 h. Overall, *R. arrhizus* (85/143, 59.4%) was most commonly associated with ROCM, while *R. microsporus* (13/38, 34.2%), and *R. homothallicus* (5/38, 13.1%) were seen mainly with pulmonary mucormycosis. *Apophysomyces* and *Saksenaea* genus were associated with GI and cutaneous mucormycosis.

Conclusions: The molecular technique utilizing semi-nested PCR, followed by Sanger sequencing was able to identify Mucorales species 81.9% of culture-negative cases. Optimized manual DNA extraction protocol is suitable for different sample types, with minimum chances of extraneous contamination and offers low cost with a shorter turnaround time (TAT).