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

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

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

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

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

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

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

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

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

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