Microbiologia Medica

Microbiologia Medica 2025; 40:13896
doi:10.4081/mm.2025.13896

Mixed fungal co-infections in post-COVID patients - role of basic microbiology techniques for wider diagnosis

Raman Sardana, Hena Butta, Ghanta Naga Sai Snigdha, Tandra Pradhan, Leena Mendiratta

Department of Microbiology, Indraprastha Apollo Hospitals, New Delhi

Summary

Background: the aim of the present study was to look for the type of fungi causing paranasal sinus/pulmonary infections in post-COVID patients, to study correlation between fungal KOH examination and fungal culture and to look for the type of fungal co-infections in these patients.

Material and Methods: this retrospective study was done on patients with post-COVID fungal infections between May 2021 to September 2021. The clinic-radiological diagnosis of fungal infection in these patients was confirmed by fungal smear KOH examination and/or fungal culture of pertinent samples. The demographic and clinical details of these patients were collected from hospital medical records.

Results: a total of 46 patients of post-COVID fungal infections were reported during study period. These were the cases of rhinosinusitis, rhino-sino-orbital and rhino-cerebral fungal infections and fungal pneumonia. Out of 46 samples 28 were suggestive of *Zygomycetes* infection and 18 were suggestive of mixed fungal infection on direct microscopic examination. On fungal culture, recovery of fungi could be done in 40 cases. The number of fungi isolated from 40 samples were 50. Different species of *Rhizopus, Aspergillus* and *Fusarium* were isolated.

Conclusions: our study depicts the occurrence of mixed fungal infections in post-COVID patients in India. These infections were caused by combination of both *Rhizopus* and *Aspergillus* as well as mixture of two different types of *Aspergillus* species. Our study underscores the meticulous utilization of basic microbiological techniques *i.e.*, fungal KOH mount and fungal culture for the optimal isolation and identification of mixed fungal infections.

Key words: mixed fungal infections, post-COVID mucormycosis, rhinosinusitis, *Rhizopus*, *Aspergillus*.

Correspondence to: Hena Butta, Department of Microbiology, Indraprastha Apollo Hospitals, New Delhi 110076, India.
E-mail: henavasdeva@yahoo.com

Introduction

The risk of opportunistic infections in Coronavirus (COVID) patients is high because of immune dysregulation and use of immuno-modulatory drugs due to the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) infection. Post-COVID secondary infections were observed worldwide and were difficult to distinguish between community-associated coinfections or Healthcare-Associated Infections (HAIs) [24]. In a review article, the frequency of occurrence of fungal co-infections in COVID patients was found to be 49.7%, 23.2%, 19.8%, 6.6%, and 0.5% in Asia, America, Europe, Africa, and Australia, respectively [23]. During the second COVID wave in India, many cases of post-COVID mucormycosis were increasingly reported in a very short time. The factors which facilitate germination of *Mucorales* spores in people with COVID include hypoxia, poorly controlled diabetes - including diabetic ketoacidosis, new onset hyperglycemia particularly due to use of steroids - high iron levels and decreased phagocytic activity of white blood cells due to immunosuppression which may be SARS-CoV-2-mediated, or steroid-mediated, or associated to to other comorbidities. Prolonged hospitalization with or without mechanical ventilators may also pose a risk factor [12]. There are several reports of post-COVID mucormycosis mainly involving paranasal sinuses leading to rhino-orbito-cerebral mucormycosis. Invasive infections with other fungi like *Aspergillus*, *Candida etc.* have also been reported to cause both pulmonary and extrapulmonary infections in post-COVID patients [13].

The spurt in cases of post-COVID fungal infections was mainly observed during the second COVID wave in India, particularly in the months from May to July 2021. Timely intervention in cases of mucormycosis is pertinent as these fungi progress very fast and cause devastating infections. Microbiology labs have a great role to play for the optimum and timely diagnosis of fungal infections. Direct microscopic examination of the specimen by fungal KOH (20-40%) wet mount examination is crucial for the timely intervention. So, the aim of the present study was to look for the type of fungi causing paranasal sinus/pulmonary infections in post-COVID patients, to study correlation between fungal KOH examination and fungal culture and to look for the type of mixed fungal co-infections in these patients.

Materials and Methods

This is a retrospective study conducted in the Department of Microbiology at Indraprastha Apollo Hospitals, New Delhi. This study was done on patients with post-COVID fungal infections between May 2021 and September 2021. Only the lab-confirmed cases were considered in the study. These were the patients in whom clinicoradiological diagnosis of Fungal infection was confirmed by fungal smear KOH examination and/or fungal culture. The demographic and clinical details of these patients were collected from hospital medical records. The permission to collect retrospective data was taken from the institute. The various samples received for fungal examination included paranasal sinus secretions, pus, tissue from paranasal sinus or rhino-cerebro-orbital region depending upon the site and extent of involvement and bronchoalveolar lavage. These samples were mainly collected through endoscope. Fungal KOH mount was made using 20% to 40% KOH with or without Dimethyl Sulfoxide (DMSO) depending upon the type of sample. Fungal culture was done on Sabouraoud's Dextrose Agar (SDA) in duplicate and incubation was done at 25ºC and 37ºC. In case of tissue samples, minimal tissue handling was done and two plates of Columbia Blood Agar were also inoculated and kept at 25ºC and 37ºC. On Fungal KOH examination, the presence of broad, sparsely septate or non septate fungal hyphae with wide angle branching was suggestive of mucormycosis; presence of thin, septate hyphae with acute angle branching was suggestive of other Hyalohyphomycetes, mainly *Aspergillus*. The identification of fungi grown in fungal culture was done by Lactophenol Cotton Blue (LPCB) mount/slide culture/Matrix Assisted Laser Desorption Ionisation Time of Flight (MALDI-TOF). All necessary biosafety and aseptic precautions were taken during processing. A correlation between fungal KOH and fungal culture was carried out. Mixed fungal co-infections were also studied in which fungal KOH and/or fungal culture were suggestive of two or more types of mycelial fungi.

Results

A total of 46 patients of post-COVID fungal infections were reported during study period. The site of infections were paranasal sinus, eye orbit, cerebrum and lungs. The samples from these patients either showed presence of fungal hyphae in the direct microscopic examination by fungal smear KOH examination or showed growth of fungi in fungal culture along with presence of fungal hyphae in direct microscopic examination. Out of 46 samples with presence of fungal hyphae on direct microscopic examination, 26 were suggestive of zygomycosis (*Supplementary Figure 1*) and 20 were suggestive of mixed fungal infection (*Supplementary Figure 2*). On fungal culture, recovery of fungi could be done in 40 cases. The number of fungi isolated from 40 samples with fungal growth were 50.

These fungi comprised of *Rhizopus* sp., *Aspergillus* sp. and *Fusarium* sp. (*Supplementary Figure 3*). The frequency of occurrence of different type of fungi is depicted in Table 1.

The frequency of occurrence of various types of fungi with respect to site of infection is depicted in Table 2. The frequency of occurrence of different *Aspergillus* sp. with respect to site of infection is depicted in Table 3.

The correlation results of fungal smear KOH examination and fungal culture are depicted in Table 4.

Table 1. Type and number of fungi in post-COVID fungal infections.

Type of fungi	Number of isolates (%)
Rhizopus oryzae complex	24 (48%)
Rhizopus homothallicus	01 (2%)
Aspergillus flavus	11 (22%)
A. fumigatus	06 (12%)
A. niger	05 (10%)
A. terreus	01 (2%)
A. nidulans	01 (2%)
Fusarium sp.	01 (2%)

Table 2. Frequency of occurrence of different fungal species with respect to site of infection.

	Rhizopus	*Aspergillus*	Mixed fungal growth (*Rhizopus* and *Aspergillus* or mixed *Aspergillus* species)	Fusarium	No growth
Rhinosinusitis	09	07	06	01	03
Rhinosinoorbital	06	-	02	-	02
Rhinocerebral	01	03	02	-	-
Fungal pneumonia	02	-	01	-	01

Table 3. Frequency of occurrence of different *Aspergillus* sp. with respect to site of infection.

	A. flavus	*A. niger*	*A. fumigatus*	*A. nidulans*	*A. terreus*
Rhinosinusitis	06	05	04	01	-
Rhino-sino-orbital	01	-	-	-	-
Rhinocerebral	03	-	02	-	01
Fungal pneumonia	01	-	-	-	-

Table 4. Correlation between direct microscopic fungal smear KOH examination and fungal culture.

Correlation between direct microscopic fungal smear KOH examination and fungal culture	Number
Number of samples with direct microscopic examination suggestive of Zygomycosis and growth of *Mucorales*	16
Number of samples with direct microscopic examination suggestive of Zygomycosis and growth of both *Mucorales* and *Aspergillus*	06
Number of samples with direct microscopic examination suggestive of Zygomycosis and growth of *Mucorales*	04
Number of samples with direct microscopic examination suggestive of mixed fungal infection and growth of both *Mucorales* and *Aspergillus*	03
Number of samples with direct microscopic examination suggestive of mixed fungal infection and growth of only *Mucorales*	02
Number of samples with direct microscopic examination suggestive of mixed fungal infection and growth of only *Mucorales*	08
Number of samples with direct microscopic examination suggestive of mixed fungal infection and growth of only *Fusarium*	01
Number of samples with direct microscopic examination suggestive of mixed fungal infection or *Mucorales* but no growth on fungal culture	06

The isolation of combination of different species of *Aspergillus* was also observed in three cases. These were mixture of *A. niger* and *A. flavus*, *A. flavus* and *A. fumigatus*, *A. fumigatus* and *A. terreus*. The species of *Aspergillus* which were isolated amongst four cases where direct microscopic examination was suggestive of zygomycosis but growth of *Aspergillus* were *A. fumigatus* (02), *A. flavus* (01) and *A. niger* (01).

Discussion

During the pandemic, opportunistic fungal co-infections in many COVID patients were reported globally. The prevalance of fungal infections after the start of the COVID pandemic in the United States increased by 9% annually between 2019-2021 [21]. The first cases of COVID Associated Pulmonary Aspergillosis (CAPA) and COVID-Associated Mucormycosis (CAM) were both reported from China [4,8]; this was followed by studies from Europe and America, reporting cases of COVID associated mucormycosis [5,15,18].

Iran was the first Asian country to report 15 cases of CAM during the first wave from April to September 2020 [17]. Other Asian countries like Pakistan, Nepal, Bangladesh also reported cases of secondary fungal infections in COVID patients [18]. India made it to the headlines due to a huge spurt in cases of CAM during the second wave of pandemic from May 2021.The central government of India declared a mucormycosis epidemic on May 10, 2021. When compared to other developed countries, the prevalence of mucormycosis in India is 80 times higher [3]. India reported >47,500 cases of CAM from May to August 2021(https://governmentstats.com/mucormycosis/index.html).This higher number of cases in India can be attributed majorly to the diabetic burden of its population. In high-income countries the site of mucor involvement is either pulmonary or disseminated, which are difficult to diagnose as the clinical and radiological pictures overlap with that of COVID, unlike the pattern observed in India and other middle and low income countries where majority of CAM cases presented with rhino-orbital mucormycosis or rhino-orbital-cerebral mucormycosis, with distinct clinical presentation. Therefore, it is likely, the true burden of CAM in high income countries is underestimated, due to lack of diagnosis [4,21].

The present study was conducted to study the epidemiology of fungal infections in post-COVID patients especially with respect to second COVID wave in India. In our study we have found that post-COVID fungal infections were caused by both *Mucorales* and *Aspergillus*. Amongst 40 patients with both fungal smear and fungal culture proven infection, the maximally isolated fungi was *Rhizopus* followed by *Aspergillus* and *Fusarium*. Mixed fungal infections by *Rhizopus* and *Aspergillus* species and also by two different *Aspergillus* species were also observed. *Rhizopus oryzae* complex (50%) was the most commonly isolated fungal species followed by *Aspergillus flavus* (18.7%). The site most commonly affected was paranasal sinus (rhinosinusitis, rhinosinoorbital, rhinocerebral) and a few cases of lung involvement (pneumonia) were also seen. In a review study of post-COVID fungal infections of maxillofacial region, out of the 81 cases reported, twenty-one cases of mucormycosis, 58 of candidiasis, and one each of aspergillosis and mixed infection were observed in the region of head and neck [10]. Sharma S. *et al.* reported 23 cases of post-COVID mucormycosis of the paranasal sinuses over the four-month study period [22]. The majority of the studies during that period focussed on mucormycosis based on radiological feature, fungal smear examination and histopathological examination [11].We want to emphasize the ocurence of mixed fungal infections in cases of post-COVID fungal infections. Like in our study, infections with both *Zygomyctes* and/or *Aspergillus* were also reported by Garg *et al*. In their study amongst 10 post-COVID patients with sinusitis and ophthalmic complications, presence of broad, sparsely septate hyphae with growth of *Mucorales* (*Rhizopus oryzae*) was seen in seven patients; thin septate hyphae with growth of *Aspergillus* sp was seen in two cases and mixed type of hyphae with growth of *Aspergillus* was seen in one case [8]. In a study by Lall M. *et al*, out of ten fungi, six were *Rhizopus*, one was *Mucor* and three were *Aspergillus* [14].

In our study, the isolation of combination of two different *Aspergillus* species were also seen in three patients. Tabarsi P. *et al*. reported a single case of COVID associated acute rhinosinusitis caused by two *Aspergillus* species *i.e. A. flavus and A. niger* [25]. COVID-associated pulmonary Aspergillosis by combination of *A. fumigatus/A. flavus* and *A. flavus/A. niger* are also reported [5,17]. There are reports of mixed fungal infections in COVID patients but mixed fungal infections with mixture of *Aspergillus* species is scarce and is an interesting area of research.

In our study, the isolation of five different types of species of *Aspergillus* were seen *i.e. A. flavus, A. niger, A. fumigatus, A. nidulans* and *A. terreus*. The reports of *A.terreus* causing fungal co-infections in COVID patients are scarce worldwide. Lall M. *et al.* reported isolation of one *A. terreus* in post-COVID fungal infections [14].Abdalla *et al*. also reported isolation of *A. terreus* in a case of pulmonary fingal infection in COVID patient.

We have also observed isolation of *Fusarium* in a case of post-COVID rhinosinusitis. The isolation of *Fusarium* sp. has been

mentioned in a case of fungal pneumonia by Casalini G. *et al.* in their narrative review on invasive fungal infections complicating COVID [2]. In their review, secondary invasive fungal infections in post-COVID patients include *Pneumocystis jirovecii* pneumonia, Cryptococcosis, Histoplasmosis, *Coccidioides* infection, pulmonary infection due to *Fusarium* spp., and pulmonary infection due to *Scedosporium* apart from *Rhizopus* and *Aspergillus* [2]. Barberis F. *et al.* reported a fatal case of invasive fusariosis due to *F. verticillioides* in an immunocompetent patient with severe COVID [1].

In our study, paranasal sinuses were involved in most of the cases (91.3%) of post-COVID fungal infections. Pulmonary fungal infections were seen in 8.6% cases. Similar finding was also observed by other authors during the current epidemic [12].

In our study, fungal growth was not observed in 13% cases despite direct demonstration of fungi on microscopic examination. This can be because of loss of viability due to prior use of antifungal before the collection of sample or may be due to oozing out of cytoplasm while handling specimen in case of *Mucorales*. Mohanti A. *et al*. found a moderate agreement between culture and KOH microscopy (Kappa=0.5) [16]. The fungal culture positivity rate in the study by Lall M. *et al.* was found to be 50%.[19] Hence, direct microscopic examination is a cost-effective and rapid tool for the early detection of presence of fungi in a clinical sample. This old-aged technique played a prudent role for the diagnosis of post-COVID fungal infections. But, at the same time fungal culture is the gold standard for the definitive differentiation and identification of the fungi and also with fungal growth antifungal susceptibility testing may be performed. Also, the addition of DMSO in KOH was found to be very useful especially in tissue samples as it significantly decreased the dissolution time of tissue and wet mount examination could be performed immediately without waiting for dissolution. Hence, fungal KOH and culture are supplementary to each other and both tests should be performed for the diagnosis of any kind of fungal infection.

In our study, the disparity between fungal KOH examination and fungal culture was mainly because of mixed fungal infections. The possibility of mucormycosis along with *Aspergillus* was there in the cases where direct microscopic examination was suggestive of *Mucorales* but culture showed growth of *Aspergillus* only. Sterile fungal culture with positive microscopic examination strongly suggests the possibility of *Mucorales*.

Molecular testing may be helpful for the identification of fungi directly from the sample especially in the scenario of negative culture despite the demonstration of fungal hyphae in the sample. But, again caution should be taken while interpreting findings of molecular results because of the likelihood of false positive results due to existence of fungal spores in the environment.

The limitation of our study is that we have not included blood stream infections caused by yeasts in our study. Also, the data with respect to serological/molecular tests for diagnosis of fungal infections was not considered. Our study mainly focusses upon mycelial fungi causing paranasal sinus and lung infections.

Conclusions

Our study depicts the occurence of mixed fungal post-COVID infections during the second COVID wave in India. Post-COVID fungal infections may be caused by combination of both Rhizopus and Aspergillus as well as mixture of two different types of *Aspergillus* species. Invasive fungal infections due to other species of fungi like *Fusarium* may also occur in post-COVID patients. Hence, broad spectrum antifungal agents should be used keeping the possibility of mixed fungal infections. Direct microscopic examination of KOH wet mount is a simple and easy method for the demonstration of fungi and it gives clue about the type of fungi causing infection including mixed infection. Fungal culture is essentially required for the definitive identification of fungi, however, there is a possibility of sterile fungal culture especially in case of *Mucorales* infection. Thus, fungal KOH and culture both are pivotal for the optimal diagnosis of infection.

References

1. Barberis F, Benedetti MF, de Abreu MS, et al. Invasive fusariosis in a critically ill patient with severe COVID-19 pneumonia: a case report. Med Mycol Case Rep 2022;35:5-8.
2. Casalini G, Giacomelli A, Ridolfo A, et al. Invasive fungal infections complicating COVID-19: a narrative review. J Fungi 2021;7:921.
3. Chander J, Singla N, Kaur M, et al. Saksenaea erythrospora, an emerging mucoralean fungus causing severe necrotizing skin and soft tissue infections—A study from a tertiary care hospital in north India. Infect Dis 2017;49:170-7.
4. Cornely OA, Alastruey-Izquierdo A, Arenz D, et al. Mucormycosis ECMM MSG Global Guideline Writing Group. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. Lancet Infect Dis 2019;19:e405-21.
5. Costache C, Botan A, Mihaila RM, et al. Mixed etiology COVID-19 associated pulmonary aspergillosis (CAPA) - a case report and brief review of the literature. Journal of Fungi 2021;7:877.
6. Devnath P, Dhama K, Tareq AM, Emran TB. Mucormycosis coinfection in the context of global COVID-19 outbreak: a fatal addition to the pandemic spectrum. Int J Surg 2021;92: 106031.
7. Dubey R, Sen KK, Mohanty SS, et al. The rising burden of invasive fungal infections in COVID-19, can structured CT thorax change the game. Egypt J Radiol Nucl Med 2022;53:18.
8. Garg R, Bharangar S, Gupta S, Bhardwaj S. Post-Covid-19 infection presenting as rhino-orbital mycosis. Indian J Otolaryngol Head Neck Surg 2022;74:3050-7.
9. Harris E. Patients with COVID-19 and fungal infections had high mortality rates. JAMA 2023;330:210-1.
10. Jain A, Taneja S. Post-COVID fungal infections of maxillofacial region: a systematic review. Oral Maxillofac Surg 2022;26:357-63.
11. Jain K, Surana A, Choudhary TS, et al. Clinical and histology features as predictor of severity of mucormycosis in post-COVID-19 patients: an experience from a rural tertiary setting in Central India. SAGE Open Med 2022;10:1-8.
12. Jose A, Singh S, Roychoudhury A, et al. Current understanding in the pathophysiology of SARS-CoV-2-associated rhino-orbito-cerebral mucormycosis: a comprehensive review. J Maxillofac Oral Surg 2021:20:373-80.
13. Kubin CJ, McConville TH, Dietz D, et al. Characterization of bacterial and fungal infections in hospitalized patients with

Coronavirus Disease 2019 and factors associated with health care-associated infections. Open Forum Infectious Diseases 2021;8:1-10.
14. Lall M, Anand KB, Shergill SPS, et al. Spectrum of fungal infections in COVID patients: The double whammy! Medical Journal Armed Forces India 2021:77:S527-9.
15. Mekonnen ZK, Ashraf DC, Jankowski T, et al. Acute invasive rhino-orbital mucormycosis in a patient with COVID-19-associated acute respiratory distress syndrome. Ophthalmic Plast Reconstr Surg 2021;37:e40-80.
16. Mohanty A, Gupta P, Arathi K, et al. Evaluation of direct examination, culture, and histopathology in the diagnosis of mucormycosis: reiterating the role of KOH mount for early diagnosis. Cureus 2021;13:e19455.
17. Nasir N, Farooqi J, Mahmood SF, Jabeen K. COVID-19-associated pulmonary aspergillosis (CAPA) in patients admitted with severe COVID-19 pneumonia: an observational study from Pakistan. Mycoses 2020;63:766-70.
18. Pakdel F, Ahmadikia K, Salehi M, et al. Mucormycosis in patients with COVID-19: a cross-sectional descriptive multicentre study from Iran. Mycoses 2021;64:1238-52.
19. Pasero D, Sanna S, Liperi C, et al. A challenging complication following SARS-CoV-2 infection: a case of pulmonary mucormycosis. Infection 2020;49:1055-60.
20. Placik DA, Taylor WL, Wnuk NM. Bronchopleural fistula development in the setting of novel therapies for acute respiratory distress syndrome in SARS-CoV-2 pneumonia. Radiol Case Rep 2020;15:2378-81.
21. Rudramurthy SM, Hoenigl M, Meis JF, et al. ECMM and ISHAM. ECMM/ISHAM recommendations for clinical management of COVID-19 associated mucormycosis in low- and middle-income countries. Mycoses 2021;64:1028-37.
22. Sharma S, Grover M, Bhargava S, et al. Post-coronavirus disease mucormycosis: a deadly addition to the pandemic spectrum. J Laryngol Otol 2021;135:442-7.
23. Seyedjavadi SS, Bagheri P, Nasiri MJ, et al. Fungal infection in co-infected patients with COVID-19: an overview of case reports/case series and systematic review. Front Microbiol 2022;13:888452.
24. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: Aa systematic review of cases reported worldwide and in India. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2021;15:102146.
25. Tabarsi P, Sharifynia S, Toutkaboni MP, et al. Mixed etiology COVID-19 associated acute rhinosinusitis caused by two Aspergillus species. Annals of Medicine and Surgery 2022;75:103365.
26. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 2020;8:475-81.

Online supplementary material:

Supplementary Figure 1. Fungal KOH examination (400X): Broad, sparsely septate branching fungal hyphae suggestive of Zygomycetes.

Supplementary Figure 2. Fungal KOH examination (400X): Two types of fungal hyphae- Later grew mixture of Aspergillus niger *and* Aspergillus flavus*.*

Supplementary Figure 3. Lactophenol Cotton Blue mount (400X): Suggestive of Fusarium *sp.*

Received: 16 April 2025; Accepted: 4 February 2026.

Authors' contributions: RS, definition of intellectual content, data analysis, manuscript editing and review; HB, concepts, design, definition of intellectual content, literature search, data acquisition and analysis, manuscript preparation; GNSS, literature search, data anlysis, manuscript editing; TP literature search, data acquisition, manuscript preparation, editing and review; LM design, definition of intellectual content, literature search, data analysis, manuscript editing and review. All the authors have read and approved the final version of the manuscript, and agreed to be held accountable for all aspects of the work.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Ethics approval and consent to participate: not applicable.

Informed consent: not applicable.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Publisher's note: all claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.