

ORIGINAL RESEARCH

Isolation and phenotypic identification of filamentous fungi in respiratory samples from a tertiary care hospital

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Received: 11 December, 2023

Accepted: 09 January, 2024

ABSTRACT

Background and Objective A rising percentage of patients with impaired immune systems are being diagnosed with fungal respiratory infections more frequently. The majority of the time, colonisation is the initial stage of a respiratory fungal infection. The objective of this study is to isolate and identify filamentous fungi from different clinically suspected respiratory samples. **Material method** This study was designed to identify filamentous fungi species from all respiratory samples. Sampling was conducted from 2021 to 2023. A total of 28 clinical isolates of molds species were obtained from the respiratory samples of both immunocompromised and immunocompetent patients. The samples were first observed under a KOH mount and inoculated on SDA. The growth was identified using standard microbiologic methods. **Result** In this study, out of all respiratory samples, 4.52% were culture-positive for mold isolates. *Aspergillus species* (21 isolates) was the most common isolate, followed by *Penicillium spp.* (2 isolates), *Rhizopus* (2 isolates), *Fusarium* (1 isolate), *Trichothecium spp.* (1 isolate), and *Acremonium spp.* (1 isolate). **Conclusion** We have concluded that the *Aspergillus* species is commonly involved in invasive respiratory infections, has emerged worldwide, and represents a serious public health concern. Early diagnosis is crucial for the successful management of patients.

Key words: Filamentous fungus, SDA, Respiratory infection, Mold

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INTRODUCTION

Respiratory fungal infections are a significant cause of death in a growing number of immunocompromised populations, including transplant recipients, patients with HIV, patients with hematologic malignancies, people with chronic pulmonary conditions, and those with autoimmune illnesses [1].

Our environment is becoming more and more infested with filamentous fungus, and the number of invasive illnesses brought on by these microbes is rising. Specifically, individuals with compromised immune systems are more susceptible to illness following filamentous fungal exposure [2] With 4.3 million fatalities annually, respiratory tract disorders account for one-third of infectious disease-associated mortality worldwide. 1. Most invasive lung fungal infections have substantial death rates (over 50%) even with therapy [3].

There are thousands of fungus spores (conidia) in the air we breathe. Following the intake of these microscopic particles, the hosts may have a range of

symptoms, from allergies to potentially fatal invasive mycoses, or they may cough up blood, experience a fever, or experience chest pain [4, 5].

A precise diagnosis of fungal pneumonia is crucial due to the many therapeutic approaches involved and the increased death rate linked to acute invasive fungal infection. Due to the lack of pathognomonic clinical features, contamination of non-invasive samples like sputum with normal commensal flora, and difficulty obtaining invasive samples like lung biopsies and transalaryngeal aspirates, the diagnosis of fungal respiratory infections is unfortunately always challenging [6].

This retrospective study was planned to study the profile of filamentous fungi in respiratory samples in a tertiary care hospital.

MATERIAL METHOD

This was a retrospective laboratory-based study conducted over a period of 3 years (January 2021 to

December 2023) in the department of microbiology at Mahatma Gandhi Memorial Medical College, Indore. All the respiratory samples, like sputum, bronchial alveolar lavage (BAL), endotracheal secretions, and pleural fluid, that were received in the mycology laboratory were included in this study. BAL, pleural fluid, and endotracheal secretions were concentrated by centrifugation at 1500–2000 g for 10 minutes. Sputum samples were treated with N-acetyl cysteine prior to inoculation on Sabouraud Dextrose Agar (SDA).

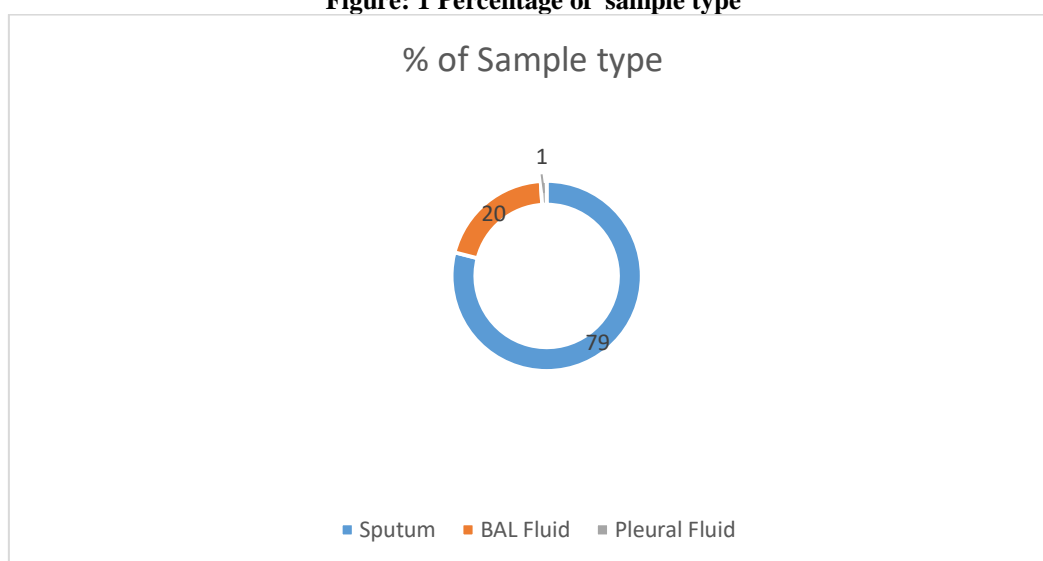
All samples were divided into two parts: the first part for the culture of the sample, and the second part for KOH-mount (10%) microscopy. 2 sets of SDA tubes (plain SDA and SDA with cycloheximide) were

inoculated, and both tubes were incubated at 25°C and 37°C. Growth on SDA media was observed on alternate days for the first week and then weekly for 4 weeks. Growth was isolated and identified using standard microbiological methods. Moulds were identified using colony morphology, microscopy, Lactophenol Cotton Blue (LPCB), and slide culture techniques.

RESULT

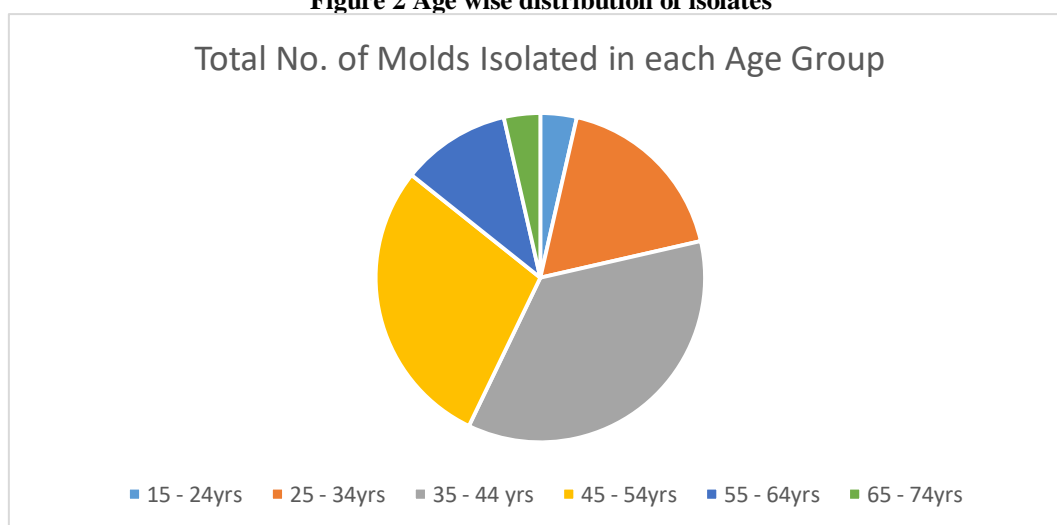
A total of 619 respiratory samples were evaluated under this study. Out of 619 respiratory samples, sputum 487 (79%) was most common followed by BAL 123 (20%) and pleural fluid 9 (1%). (Fig1)

Figure: 1 Percentage of sample type



Out of 619, 28 (4.52%) were culture positive for molds. The most common age group affected was 30 to 50 years(Fig 2). Males were more commonly affected than females with 53.57% and 46.43% respectively.

Figure 2 Age wise distribution of isolates



Isolation rates from sputum were highest (22 samples; 78.57%), followed by BAL (6 samples; 21.42%). A total of 2 samples were both KOH and culture-

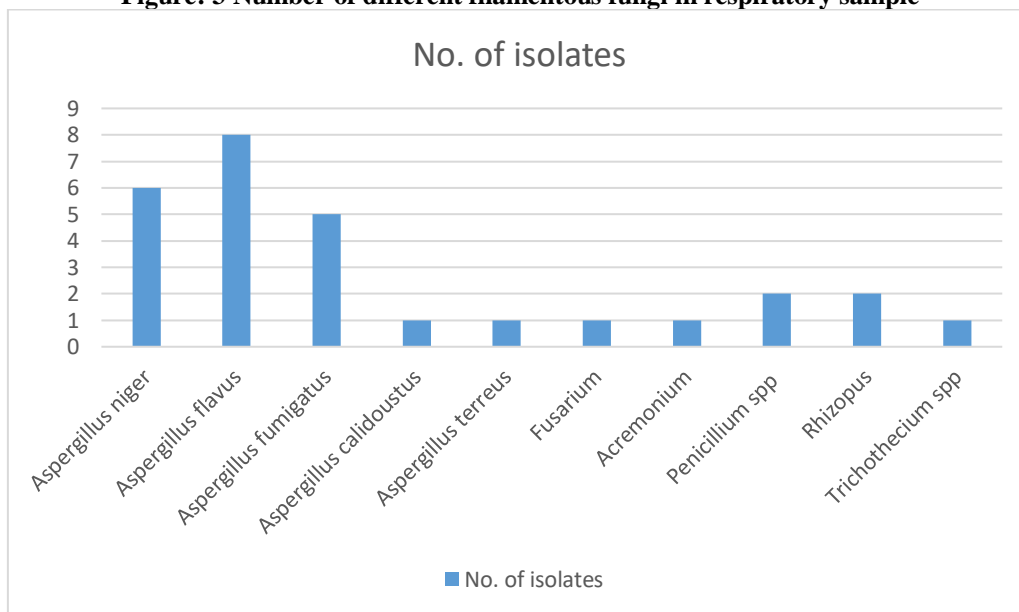
positive, but the rest of the samples were positive only by the culture method.

In sputum samples, *Aspergillus spp.* is the most common isolate, accounting for 75% (17 isolates). Among the *Aspergillus spp.*, *Aspergillus flavus* contributed 8 isolates, followed by *Aspergillus niger* (4 isolates), *Aspergillus fumigatus* (4 isolates), *Aspergillus terreus* (1 isolate), *Rhizopus* (1 isolate),

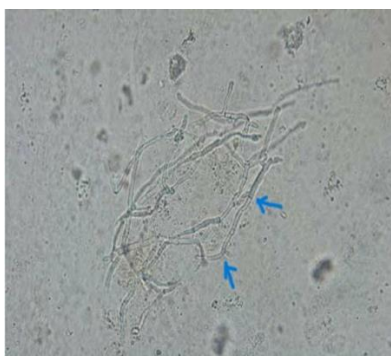
Trichothecium spp., *Acremonium spp.* (1 isolate), and *Penicillium spp.* (2 isolates).

In BAL samples, there were 2 isolates of *Aspergillus niger*, 1 isolate of *Aspergillus fumigatus*, 1 isolate of *Aspergillus calidoustus*, 1 isolate of *Fusarium* and 1 isolate of *Rhizopus*.

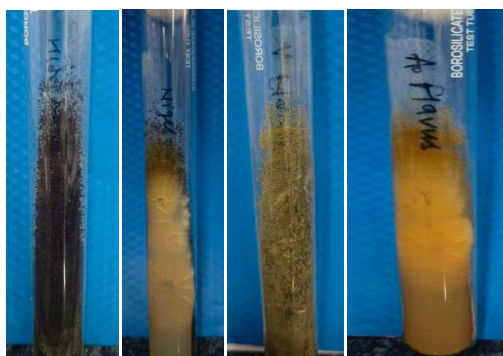
Figure: 3 Number of different filamentous fungi in respiratory sample



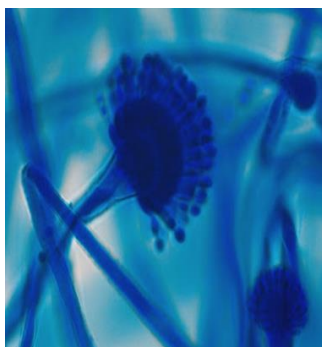
Overall, in respiratory samples, *Aspergillus species* (21 isolates) was the most common isolate, followed by *Penicillium spp.* (2 isolates), *Rhizopus* (2 isolates), *Fusarium* (1 isolate), *Trichothecium spp.* (1 isolate), and *Acremonium spp.* (1 isolate) figure 3.



KOH mount showing septate hyphae



Growth on SDA tube (A) *Aspergillus niger* (B) *Aspergillus flavus*



LPCB showing *Aspergillus flavus*.

DISCUSSION

Filamentous fungi have always been a matter of concern in the medical field, but nowadays, due to an increase in risk factors, they are slowly but surely emerging as a dangerous health threat worldwide.

This retrospective study details the three-year burden of filamentous fungus in respiratory tract infections in a tertiary care facility. Methods based on culture were mostly used for diagnosis. During this time, *Aspergillus* species were the most often isolated genus.

In this study, out of all respiratory samples, 4.52% were culture-positive for mold isolates, and among all respiratory samples, sputum samples (78.57%) showed the highest positivity rate. This was similar to the study conducted by Biswas *et al.* [6], where sputum samples showed 65% positivity.

In the present study, males were more affected, which is like many other studies related to respiratory tract infections, which is similar to the study conducted by Roohani *et al.* [7]

Aspergillus species (75%) were the most predominant species isolated from all respiratory samples, similar to the study by Biswas *et al.* *Aspergillus* species were the most commonly isolated fungal pathogens [6].

Among *aspergillus* species, *aspergillus flavus* was most commonly isolated in this study, which is similar to the results shown by Hedayati MT *et al.* [8, 9]

According to the results of the present research, *Aspergillus* species are thought to be the primary cause of respiratory tract mycosis and may be accountable for a considerable amount of morbidity and mortality. This study offers comprehensive insights on the range of fungal infections in tertiary care hospitals and may serve as an opportunity for further study into this important field of clinical research.

CONCLUSION

Globally, filamentous mycoses are gradually gaining alarm as a health hazard. New risk factors and therapies emerge as life expectancy rises, and the population gradually changes to become a more vulnerable target for those kinds of infections. In summary, thorough research on the real burden, regional distribution, and underlying risk factors of individuals with mold-related respiratory mycoses is still lacking. Clinical suspicion and wider recognition

of diseases linked to fungi by microbiologists and clinicians would enhance the therapeutic experience and, in turn, lead to the development of improved treatment plans.

Source of funding: none

Conflicts of interest: none declared

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