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CHANGING TRENDS IN RESPIRATORY FUNGAL INFECTIONS AMONG IMMUNOCOMPETENT AND IMMUNOCOMPROMISED PATIENTS: SPECTRUM OF FUNGAL PATHOGENS AND THEIR ANTIFUNGAL SUSCEPTIBILITY

Clinical Microbiology

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ABSTRACT

Introduction: Respiratory fungal infections are increasingly recognized as important causes of morbidity and mortality, particularly among immunocompromised individuals. The epidemiology of these infections is evolving with changing host factors, increasing prevalence of HIV infection, diabetes mellitus, malignancies, and widespread antifungal use. This study aimed to determine the spectrum of respiratory fungal pathogens among immunocompetent and immunocompromised patients and evaluate their antifungal susceptibility patterns. **Materials and Methods:** A prospective observational study was conducted in the Department of Microbiology, Government Medical College, Amritsar, from July 2024 to December 2025. A total of 217 patients presenting with respiratory symptoms were enrolled. Patients were categorized into immunocompetent (n=105) and immunocompromised (n=112) groups. Respiratory samples were subjected to direct microscopy, fungal culture, species identification, galactomannan antigen detection, and antifungal susceptibility testing according to CLSI guidelines. **Results:** Candida albicans was the predominant pathogen (61.4%), followed by Aspergillus flavus (15.7%), Aspergillus fumigatus (5.7%), Fusarium (4.2%), Mucor (4.2%), Candida parapsilosis (2.8%), Candida tropicalis (2.8%) and Aspergillus niger (2.8%). Culture positivity was significantly higher among immunocompromised patients (43.8%) than immunocompetent patients (20%). All Candida isolates were sensitive to caspofungin, while fluconazole resistance was observed in 55.8% of Candida albicans isolates. Amphotericin B and caspofungin demonstrated excellent activity against Aspergillus isolates. **Conclusion:** Respiratory fungal infections are more common among immunocompromised individuals, with Candida albicans and Aspergillus species being the predominant pathogens. Increasing resistance to azoles highlights the importance of routine antifungal susceptibility testing and antifungal stewardship. Early diagnosis using culture and galactomannan assay can improve patient outcomes.

KEYWORDS

Respiratory Fungal Infections, Candida Albicans, Aspergillus, Immunocompromised Host, Galactomannan, Antifungal Susceptibility, HIV

INTRODUCTION

Respiratory fungal infections have emerged as an important cause of morbidity and mortality worldwide, particularly among individuals with impaired immunity. Respiratory tract infections account for a substantial proportion of infectious disease-related deaths globally, and fungal pathogens are increasingly recognized as significant contributors to this burden. The rising incidence of fungal respiratory infections is largely attributed to the expanding population of immunocompromised patients, including those with Human Immunodeficiency Virus (HIV) infection, diabetes mellitus, malignancies, organ transplantation, chronic lung diseases, and individuals receiving corticosteroids or other immunosuppressive therapies. [1]

The respiratory tract serves as the primary portal of entry for numerous pathogenic fungi. Inhalation of airborne fungal spores is common; however, effective innate and adaptive immune responses usually prevent disease development. Disruption of these defense mechanisms may result in fungal colonization, allergic manifestations, chronic pulmonary disease, or invasive fungal infections.[2] Chronic lung conditions such as chronic obstructive pulmonary disease (COPD), bronchiectasis, and previous tuberculosis have also been recognized as important predisposing factors for respiratory mycoses.[3]

A wide variety of fungal pathogens are implicated in respiratory infections. Opportunistic fungi commonly encountered in immunocompromised hosts include species of *Candida*, *Aspergillus*, Mucorales, *Cryptococcus neoformans*, *Pneumocystis jirovecii*, *Trichosporon*, and *Fusarium*.[1,4] In recent years, it has become increasingly evident that fungal respiratory infections are not restricted to immunocompromised individuals and may also occur in immunocompetent hosts with underlying pulmonary disorders or environmental exposures.[5]

Among yeast pathogens, *Candida albicans* remains the predominant species causing respiratory fungal infections; however, infections due to non-*albicans Candida* species are increasing worldwide.[6] These species are clinically important because they often exhibit reduced susceptibility to commonly used antifungal agents. Similarly, *Aspergillus* species are important respiratory pathogens capable of causing a spectrum of diseases ranging from allergic bronchopulmonary aspergillosis and aspergilloma to invasive pulmonary aspergillosis, particularly in immunocompromised patients.[7]

The clinical manifestations of respiratory fungal infections are often nonspecific and mimic bacterial or viral respiratory illnesses, leading to delayed diagnosis and treatment.[8] Conventional diagnostic methods such as direct microscopy and fungal culture remain the cornerstone of diagnosis; however, newer molecular and serological techniques have improved the early detection of invasive fungal infections.[9]

The emergence of antifungal resistance among *Candida* and *Aspergillus* species has become a growing concern due to the widespread use of antifungal agents.[10,11] Antifungal susceptibility testing plays a crucial role in guiding therapy, monitoring resistance trends, and improving clinical outcomes.[12] Therefore, the present study was undertaken to evaluate the changing spectrum of respiratory fungal pathogens among immunocompetent and immunocompromised patients and to determine their antifungal susceptibility patterns.

MATERIALS AND METHODS

A prospective observational study was conducted in the Department of Microbiology, Government Medical College, Amritsar, over a period of one and a half years from July 2024 to December 2025. A total of 217 patients presenting with respiratory symptoms and attending the ART clinic or the Department of Chest and Tuberculosis were included. Patients were classified as:

Inclusion Criteria

Patients presenting with respiratory symptoms such as cough and dyspnoea, along with constitutional symptoms including fever, headache, loss of appetite, and weight loss, were included. Patients with comorbidities such as tuberculosis, COPD, pneumonia, ARDS, diabetes mellitus, and malignancies were enrolled. HIV-positive patients and/or those with a neutrophil count <500 cells/µL were classified as immunocompromised, while patients with a neutrophil count >500 cells/µL were considered immunocompetent. Written informed consent was obtained from all participants.

Exclusion Criteria

Patients without respiratory and constitutional symptoms suggestive of respiratory fungal infection and those receiving antifungal therapy were excluded from the study.

Sample Collection

Expectorated sputum, induced sputum, bronchoalveolar lavage

(BAL), endotracheal aspirates, pleural fluid, and blood samples were collected from patients and transported promptly to the microbiology laboratory. All specimens were processed within one hour of collection to ensure optimal recovery of pathogens, while serum samples were preserved at −20°C until further analysis.

Laboratory Investigations[13,14]

Direct Microscopy: Clinical sample were stained using Gram stain, Giemsa stain, KOH mount, calcofluor white stain, and India ink.

Culture on Sabouraud Dextrose Agar (SDA): Following inoculation and incubation, all culture media were inspected daily during the first week for evidence of fungal growth and subsequently on alternate days for a total duration of three weeks. Fungal isolates were identified based on their colony morphology and microscopic features using conventional mycological methods and standard identification procedures described in medical mycology literature.

Identification of Yeast and Mould Isolates: Yeast isolates were identified initially based on their colony morphology and were further characterized using germ tube testing, microscopic morphology on corn meal agar, growth characteristics on chromogenic Candida agar, urease activity, carbohydrate fermentation tests, and carbohydrate assimilation studies performed on yeast nitrogen base agar. Mould isolates were identified and speciated by evaluating their macroscopic colony features, microscopic morphology observed in lactophenol cotton blue mounts, and characteristic structures demonstrated by slide culture techniques.

Detection of Aspergillus Galactomannan Antigen: It was done using Human Platelia TM Aspergillus ELISA (Shanghai Coon Koon Biotech Co., Ltd).

Antifungal Susceptibility Testing: The Clinical and Laboratory Standards Institute (CLSI) guideline M44-A2 was strictly followed for antifungal susceptibility testing of yeasts using the disc diffusion method. The antifungal agents evaluated included amphotericin B, fluconazole and caspofungin. For mould susceptibility testing, the CLSI M38-A2 broth microdilution protocol was followed. The antifungal agents tested against mould isolates were amphotericin B, voriconazole, and caspofungin.[15,16,17]

Statistical Analysis

Data were analyzed using SPSS version 24. A p-value <0.05 was considered statistically significant.

RESULTS

Most patients belonged to the 40–60 years age group with mean age of 53 year. There was marked male predominance (65.4%) with Male: female ratio of 1.9: 1. Out of the total 217 patients, 112 were immunocompromised and 105 were immunocompetent.

Distribution of Patients According to Immune Status and Clinical Diagnosis (n = 217)

Diagnosis	Immuno-compromised	Immuno-competent	Total
Pulmonary Tuberculosis	22	50	72
Pneumonia	10	14	24
COPD	7	21	28
Acute Respiratory Distress Syndrome	0	9	9
Bronchiectasis	4	9	13
HIV	69	0	69
Total	112	105	217
P value	p < 0.001		

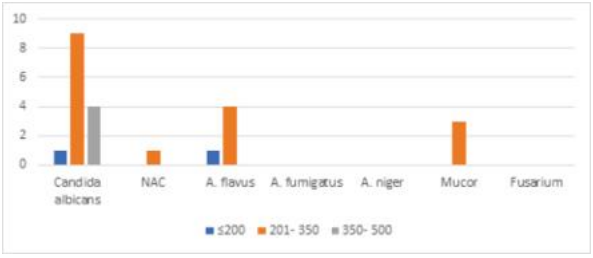
Among 217 patients, fungal growth was observed in 70 cases which includes *Candida albicans* – 43 (61.4%), *Aspergillus flavus* – 11 (15.7%), *Aspergillus fumigatus* – 4 (5.7%), *Non-albicans Candida* – 4 (5.7%), *Fusarium spp.* – 3 (4.2%), *Mucor spp.* – 3 (4.2%) and *Aspergillus niger* – 2 (2.8%)

Association Between Immune Status and Culture Findings

Culture Finding	Immuno-compromised	Immuno-competent	Total
Candida albicans	27	16	43
Aspergillus flavus	8	3	11
Aspergillus fumigatus	3	1	4
Aspergillus niger	2	0	2
NAC	3	1	4
Fusarium	3	0	3
Mucor	3	0	3
P value = < 0.001			

Relationship with CD4 count in HIV Positive patients: Fungal culture positivity in HIV-positive patients was more in those with CD4 counts of 201–350 cells/mm³ (17 cases), followed by 350–500 cells/mm³ (4 cases) and ≤200 cells/mm³ (2 cases). *Candida albicans* was the most common isolate across all CD4 groups, while other fungi such as *Aspergillus flavus* and Mucor were mainly seen in the 201–350 cells/mm³ group.

Distribution of Culture Isolates According to CD4 Count in HIV Positive Patients



Antifungal susceptibility pattern: All *Candida* species were 100% sensitive to caspofungin while 26 (55.31%) were resistant to fluconazole when performed by disc diffusion method. Among moulds AFST was performed using Broth microdilution method where, Amphotericin B and Caspofungin represented good susceptibility, while voriconazole showed resistance in 3 (27.3%) isolates of *Aspergillus flavus* and 1 (25%) isolates of *Aspergillus fumigatus*.

DISCUSSION

Respiratory tract infections can be caused by a diverse group of microorganisms, including bacteria, viruses, and fungi. In recent years, there has been a noticeable rise in the detection of fungal infections. This increase is primarily linked to the growing number of susceptible individuals, especially those receiving immuno-suppressive therapy, patients undergoing bone marrow or solid organ transplantation, and those living with conditions such as HIV infection, tuberculosis, and cystic fibrosis.

Among immunocompromised patients, *Candida albicans* was the predominant isolate, accounting for 27 (55.10%) cases, followed by *Aspergillus flavus* in 8 (16.32%) cases. *Aspergillus fumigatus*, *Fusarium*, and *Mucor* species were each isolated in 3 (6.12%) cases. In addition, *Aspergillus niger* and *Candida tropicalis* were identified in 2 (4.08%) cases each, while *Candida parapsilosis* was recovered from 1 (2.04%) case.

Similarly, *Candida albicans* was the most frequently isolated fungus among immunocompetent patients, accounting for 16 (76.19%) cases. This was followed by *Aspergillus flavus* in 3 (14.28%) cases, whereas *Aspergillus fumigatus* and *Candida parapsilosis* were each detected in 1 (4.76%) case. However, fungal diversity was greater among immunocompromised individuals, reflecting their increased susceptibility to a broader range of opportunistic fungal pathogens.

The findings are consistent with studies by Rafat et al., Latgé et al.[1,18], which reported similar distributions of fungal pathogens. The significantly higher prevalence of fungal isolation among immunocompromised patients highlights the critical role of host immunity in susceptibility to fungal infections. HIV infection and tuberculosis constituted the most important underlying risk factors in our population.

Diabetes mellitus was the most common risk factor identified, followed by steroid use and hypertension. Diabetes predisposes individuals to fungal infections by impairing neutrophil function and cellular immunity, consistent with the findings of Brown et al.[19] Similarly, corticosteroid therapy promotes immunosuppression and increases the risk of fungal infections. The contribution of smoking and alcoholism observed in the present study is also supported by Chakrabarti et al.[20], who reported these lifestyle factors as important contributors to fungal disease burden.

Candida species, particularly *Candida albicans*, emerged as the most common isolate across all CD4 categories in this study. Among filamentous fungi, *Aspergillus flavus* and *Mucor* species were

predominantly observed in patients with CD4 counts between 201–350 cells/mm³, indicating susceptibility even at intermediate levels of immunosuppression. This observation aligns with findings by Roohani AH et al.[21], who reported that opportunistic mould infections are not restricted to profoundly immunocompromised patients but may also occur in those with moderate immune dysfunction. The presence of *Aspergillus* species in this CD4 range underscores the importance of early screening and diagnostic vigilance.

The present study demonstrates a strong correlation between serum galactomannan (GM) assay and conventional diagnostic modalities for pulmonary aspergillosis, while also highlighting the known limitations of antigen-based testing. Our findings align with those of Hope et al.[22], who emphasized that GM detection serves as an early marker of invasive aspergillosis, often preceding culture positivity. Therefore, Galactomannan assay proved valuable in the early diagnosis of pulmonary aspergillosis and showed strong correlation with culture and smear positivity. The findings support its incorporation into routine diagnostic algorithms.

In the present study, a substantial degree of resistance to fluconazole was detected, with *Candida albicans* exhibiting 55.81% of resistance and *Non-albicans Candida* showing 50% resistance. Comparatively, voriconazole demonstrated greater antifungal activity, with 60.46% of *Candida albicans* isolates and 50% of *Non-albicans Candida* isolates being sensitive. These findings indicate that although voriconazole appears to be a more effective therapeutic option than fluconazole, the presence of significant resistance highlights the need for judicious use and ongoing surveillance of antifungal susceptibility patterns.

Among *Aspergillus* isolates, all *Aspergillus flavus* (n=11), *Aspergillus fumigatus* (n=4), and *Aspergillus niger* (n=2) were fully susceptible to amphotericin B and caspofungin, indicating excellent efficacy of these agents. Voriconazole demonstrated good activity, with sensitivity rates of 72.7% in *A. flavus*, 75% in *A. fumigatus*, and 100% in *A. niger*. However, resistance was observed in 27.3% of *A. flavus* and 25% of *A. fumigatus* isolates, suggesting the emergence of azole resistance. These findings are in agreement with studies by Maiken Cavling Arendrup and Emily L. Berkow[11, 12], which reported similar trends in antifungal resistance.

CONCLUSION

This study underscores the significant burden of respiratory fungal infections, particularly among immunocompromised individuals, while also highlighting their occurrence in immunocompetent patients. HIV infection, diabetes mellitus, and corticosteroid therapy emerged as important predisposing factors. *Candida albicans* remained the predominant pathogen, followed by *Aspergillus* species, with an increasing contribution from non-*albicans Candida* species, indicating evolving epidemiological trends.

The study also demonstrated the value of the galactomannan assay as a rapid and effective adjunctive diagnostic tool for the early detection of pulmonary aspergillosis, facilitating timely therapeutic intervention. Antifungal susceptibility testing revealed concerning levels of resistance to fluconazole and emerging resistance to voriconazole among both yeast and mould isolates, emphasizing the growing challenge of antifungal resistance. In contrast, amphotericin B and caspofungin retained excellent activity against most isolates.

Overall, these findings highlight the need for early and accurate diagnosis, routine antifungal susceptibility testing, and strengthened antifungal stewardship programs. Rational antifungal use, coupled with increased clinician awareness and the integration of advanced diagnostic modalities, is essential to optimize patient outcomes and curb the emergence of antifungal resistance.

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