

The Spectrum of Fungal Isolates in Respiratory Samples in a Tertiary Care Centre: A Retrospective Study

ANN MARIA VARGHESE¹, SUSHMA PEDNEKAR², VAISHALI WABALE³, SAI WYAWAHARE⁴, RAJESH KARYAKARTE⁵

ABSTRACT

Introduction: Fungal pneumonia is a pulmonary infection caused by endemic or opportunistic fungi. Its development is strongly associated with predisposing factors such as immunocompromised states like Human Immunodeficiency Virus (HIV) infection, malignancy, organ transplantation, or corticosteroid therapy, underlying chronic diseases, and hypersensitivity or allergic responses to fungal organisms. This occurs following the inhalation of spores, inhalation of conidia, or the reactivation of a latent infection. In most cases, colonisation is the first step in the progression to pulmonary fungal infection.

Aim: To determine the spectrum of fungal pathogens in respiratory samples of symptomatic patients in a tertiary care centre.

Materials and Methods: A retrospective study was conducted over a period of 18 months, starting from January 2024 till June 2025, in the Department of Microbiology, BJ Government Medical College and Sassoon Hospitals, Pune, Maharashtra, India. A total of 560 respiratory samples of patients with symptoms like fever, cough, breathlessness, facial swelling etc. received in the Mycology department, were included in the study. Samples were processed for direct microscopy using 10% KOH (Potassium

hydroxide) and for culture on Sabouraud's Dextrose Agar (SDA). The growth on SDA was identified using conventional fungal identification methods including Gram staining, germ tube test, inoculation on CHROM agar and on Corn meal agar for Yeasts and for Moulds, Lactophenol Cotton Blue (LPCB) mount and slide culture. All data received were noted in Microsoft Excel and statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 23.0.

Results: A total of 560 respiratory samples were received during the study period. Out of these, 122 samples were positive for fungal isolates. The most common isolated species was *Candida* species 93 (76.22%), followed by *Aspergillus* species 14 (11.47%). Among *Candida*, the most common was *Candida albicans* 56 (60.2%), and among *Aspergillus*, the most common was *Aspergillus fumigatus* 6 (4.92%) and *Aspergillus flavus* 6 (4.92%).

Conclusion: *Candida albicans* was the most common fungal species isolated from symptomatic patients. The present study indicates that pulmonary fungal infection is a significant concern among patients with respiratory diseases, highlighting the critical importance of early diagnosis.

Keywords: *Aspergillus fumigatus*, *Candida albicans*, Immunocompromised host, Pulmonary mycoses, Respiratory specimens

INTRODUCTION

Respiratory infections continue to be a major global health challenge and remain the leading infectious cause of death worldwide. Fungal pneumonia is an infectious process in the lung caused by one or more endemic or opportunistic fungi. Unfortunately, the lack of pathognomonic clinical features, contamination of non-invasive specimens like sputum with normal commensal flora, and difficulties in obtaining invasive specimens like lung biopsies and trans-laryngeal aspirates make it difficult to diagnose fungal respiratory infections. Fungal infections in patient care settings are not well documented in India, with contradictory findings [1]. Recent Global Burden of Disease analyses indicate that lower respiratory infections account for millions of deaths annually, while pulmonary fungal infections remain substantially under-recognised despite causing an estimated 5.62 million cases and over 45,000 deaths globally in 2021 [2]. The majority of invasive chronic respiratory fungal infections are linked to significant death rates of more than 50% even with therapy [3]. Invasive fungal infections have become frequent in the healthcare environment [4]. Across the globe, respiratory and systemic fungal infections are becoming more significant issues in infectious diseases. Airborne fungi constitute a major component of atmospheric bioaerosols, displaying considerable diversity and temporal variability, as demonstrated by molecular analyses of outdoor air samples [5]. Fungal colonisation, allergies, and infections of the airways and respiratory tract make

up pulmonary fungal illnesses. In most cases, colonisation is the initial stage leading to respiratory fungal infection [6]. The number of people diagnosed with pulmonary fungal diseases has increased over the past few decades, primarily due to the growing number of people at risk, including those undergoing Solid Organ Transplantation (SOT), receiving immunosuppressive therapy, and having HIV, tuberculosis, or cystic fibrosis [6]. The occurrence of candidaemia, systemic aspergillosis, cryptococcosis, and zygomycosis in India has significantly increased, along with the rise of new fungal infections [7].

Due to the numerous treatment options available and the higher mortality risk associated with acute invasive fungal infection, an accurate diagnosis of fungal pneumonia is essential. Unfortunately, diagnosing fungal respiratory infections is always difficult due to the absence of pathognomonic clinical features, contamination of non-invasive specimens like sputum with normal commensal flora, and challenges in obtaining invasive specimens like lung biopsies and trans-laryngeal aspirates [8]. There is a lack of data from India regarding fungal infections in the patient care environment, showing inconsistent results [1,9]. In order to collect data on this subject, surveillance studies on fungal infections and colonisation must be carried out in a variety of healthcare facilities around the country.

This study aimed to characterise the spectrum of fungal isolates recovered from respiratory specimens of patients with suspected respiratory fungal infections in a tertiary care hospital.

MATERIALS AND METHODS

This retrospective study was conducted in the Department of Microbiology, BJ Government Medical College and Sassoon Hospitals, Pune, Maharashtra, India. Clinical and laboratory records from January 2024 to June 2025 were reviewed. Data extraction and statistical analysis were carried out from July 2025 to August 2025. The study was approved by the Institutional Ethics Committee (IEC No- ND-0825273-273). As the study involved retrospective analysis of laboratory data, informed consent was obtained. For eligible cases included in the study, written informed consent was obtained from patients or their legally authorised representatives prior to inclusion of their laboratory data in the analysis. All patient identifiers were removed to ensure confidentiality.

Sample size: A universal sampling approach was adopted for this retrospective study. All respiratory specimens received in the Mycology laboratory of the Department of Microbiology, BJ Government Medical College and Sassoon General Hospitals, Pune during the study period (January 2024 to June 2025) that fulfilled the inclusion criteria were included in the analysis [10].

Inclusion criteria: All respiratory samples like Sputum, Broncho-Alveolar Lavage (BAL), endotracheal secretions, pleural fluid, nasal crest, nasal swab and sinus tissue etc., received in Mycology department from OPD and IPD patients of any age, were included in the study.

Exclusion criteria: Samples other than the respiratory ones and respiratory samples in which a non-fungal pathogen had been identified were excluded from the study.

Study Procedure

Deep-coughed sputum specimens obtained from patients showing clinical signs of fungal respiratory tract infections were part of this study, and at least three sputum samples were collected. Sputum specimens were collected in a clean, wide-mouthed receptacle. Before obtaining the sputum sample, patients were instructed to rinse their mouth with distilled water to prevent contamination from the commensal flora of the oral cavity [11]. The Bartlett scoring method was used to avoid salivary contamination in sputum samples; samples with a score of zero or lower, since they are not relevant samples, were discarded [12]. Specimens such as BAL, endotracheal secretions, and pleural fluids were concentrated through centrifugation at 1500 g-2000 g for a duration of 10 minutes [8].

The received samples were divided into two parts, one for direct microscopy using 10% KOH and the second part for culture on SDA (HiMedia Pvt., Ltd., Mumbai). To visualise the presence of fungal elements or any budding yeast cells, direct microscopic examination was done by using 10% KOH for all the samples. Gram staining was done to identify Gram-positive yeast cells. All respiratory samples received were cultured by inoculating in duplicates on SDA both with antibiotics (Chloramphenicol and Cycloheximide) and without antibiotics. Culture tubes were incubated at 25°C and 37°C and examined for up to four weeks for the growth of any fungus. SDA tubes were examined for growth every day during first week and once every two days thereafter up to four weeks.

The yeast isolates were processed and identified by Gram staining, germ tube test, inoculation on CHROM agar (HiMedia Pvt., Ltd., Mumbai) and morphologically on cornmeal agar (HiMedia Pvt., Ltd., Mumbai). Mould isolates were identified by conventional methods like colony morphology, LPCB mount and slide culture.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 23.0. All the data obtained were collected and entered into a Microsoft Excel sheet version 2019.

Descriptive analysis was performed to determine the frequency and percentage distribution of fungal isolates, specimen types, and patient characteristics.

RESULTS

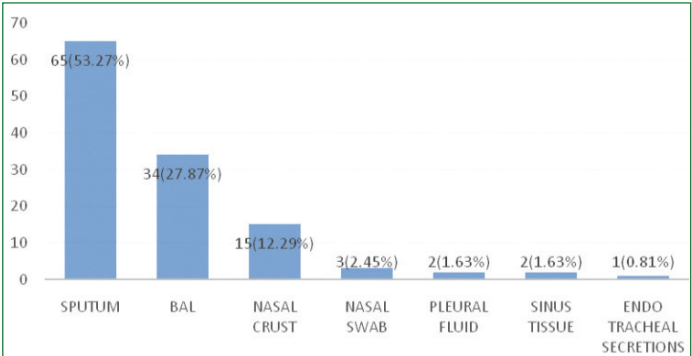
A total of 560 respiratory samples, including sputum, BAL, nasal crust, nasal swab, pleural fluid, sinus tissue and endotracheal secretions were received during the study period. Out of the total 560 samples, 122 samples were positive for fungal isolates which included both yeasts and moulds. Fungal isolates were predominantly recovered from patients aged 41-50 years, with a mean age of 44.91 years [Table/Fig-1].

Age group (years)	n (%)
0-10	3 (2.45)
11-20	8 (6.55)
21-30	14 (11.47)
31-40	18 (14.75)
41-50	32 (26.22)
51-60	26 (21.31)
61-70	14 (11.47)
71-80	7 (5.73)

[Table/Fig-1]: Showing age distribution of study participants (N=122).

There was a male predominance seen, 84 (68.85%) being male patients and 38 (31.15%) being female patients. Majority of samples positive for fungal pathogens were received from in-patient department 74 (60.66%), rather than from outpatient department 48 (39.34). Among IPD patients, maximum samples were received from Respiratory medicine ward 38 (51.35%), followed by medical ICU patients 20 (27.03%).

Sputum was the predominant specimen (53.28%), followed by BAL (27.87%), indicating that most fungal-positive samples were respiratory specimens. Other specimen types were less frequently represented [Table/Fig-2].

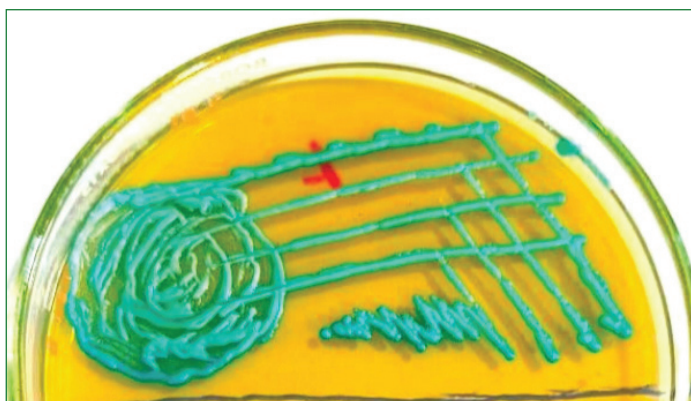


[Table/Fig-2]: Showing various samples received during the study (N=122).

BAL: Broncho-alveolar lavage

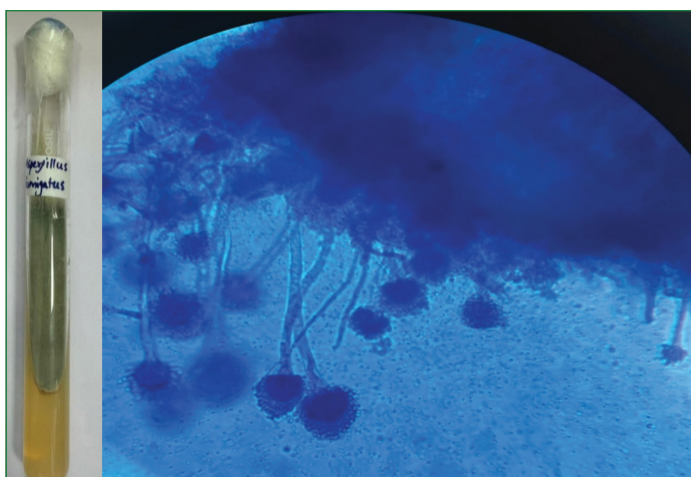
The chromogenic medium demonstrated characteristic growth of *Candida albicans*, supporting its presumptive identification based on the typical colony colour and appearance [Table/Fig-3]. Among the 122 positive samples, a total of 93 *Candida* species (76.22%) were isolated. Among the 93 *Candida* species, the most isolated *Candida* species was *Candida albicans* 56 (60.21%), followed by *Candida tropicalis* 17 (18.27%), *Candida parapsilosis* 10 (10.75%), *Candida glabrata* 9 (9.67%), and *Candida krusei* 1 (1.07%). *Trichosporon* species 1 (0.81%) was also isolated from a single sample, which was reported separately.

The *Candida albicans* species was mainly isolated from samples like sputum, broncho alveolar lavage and nasal crust. The patients from whom *C. albicans* was isolated from respiratory samples also had clinical features suggestive of oral candidiasis, which supported the significance of the isolates. Other Non-*albicans Candida* were mainly isolated from Sputum and Broncho alveolar lavage samples.

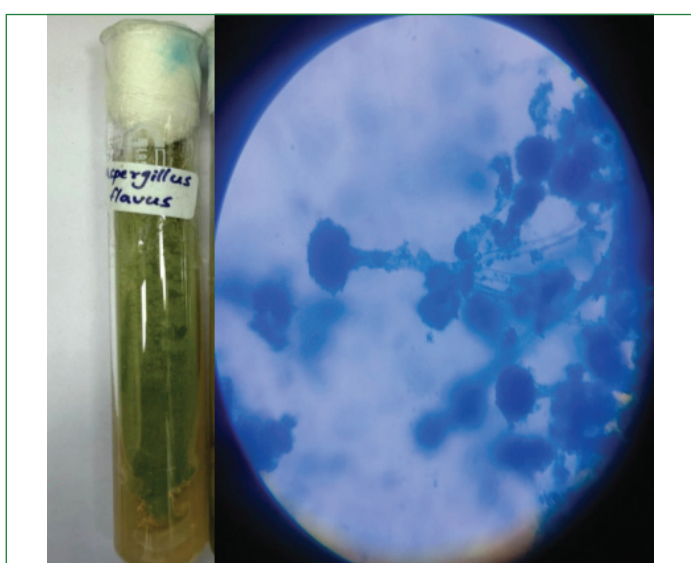


[Table/Fig-3]: Chrome agar showing growth of *Candida albicans*.

Among moulds, the most common species isolated were *Aspergillus fumigatus* 6 (4.91%) and *Aspergillus flavus* 6 (4.91%). *Aspergillus fumigatus* showed characteristic uniseriate phialides on the upper two-thirds of the vesicle [Table/Fig-4], whereas *Aspergillus flavus* demonstrated biseriate phialides covering most of the vesicle [Table/Fig-5]. Only one isolate of *Aspergillus terreus* was obtained [Table/Fig-6] which showed biseriate phialides forming only on the upper half of vesicle.

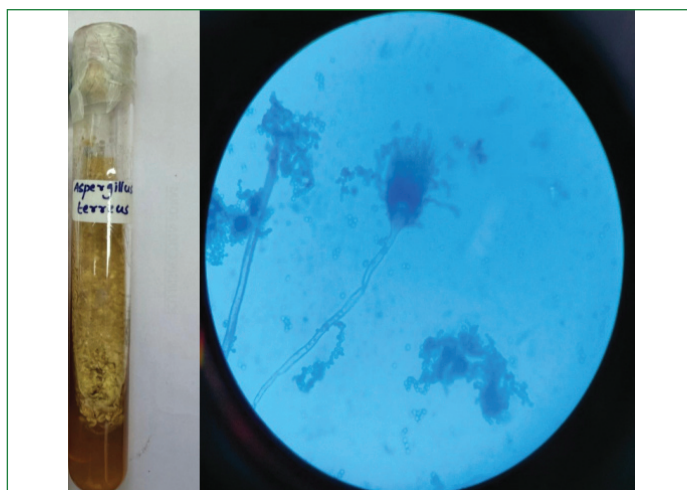


[Table/Fig-4]: Showing SDA growth and LPCB mount of *Aspergillus fumigatus* with uniseriate phialides forming only on the upper two-thirds of the vesicle (LPCB x400).

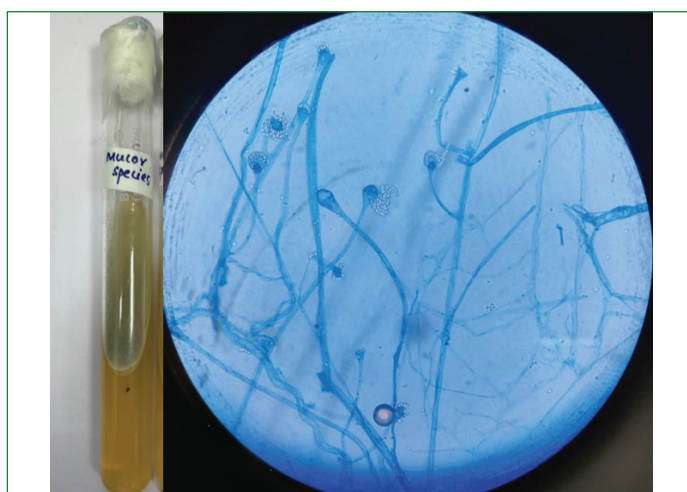


[Table/Fig-5]: Showing SDA growth and LPCB mount of *Aspergillus flavus* with biseriate phialides formed over most of the vesicle (LPCB x400).

The second most common mould group isolated was mucorales (N=9), comprising *Rhizopus* species (4.09%, N=5) and *Mucor* species (3.27%, N=4). *Mucor* species showed broad aseptate hyphae with long, branched sporangiophores bearing terminal, round sporangia and absence of rhizoids [Table/Fig-7].



[Table/Fig-6]: Showing SDA growth and LPCB mount of *Aspergillus terreus* with biseriate phialides, metulae and phialide being equal in length, forming only on the upper half of vesicle (LPCB x400).



[Table/Fig-7]: Showing SDA growth and LPCB mount of *Mucor* species with broad aseptate hyphae. Long and branched sporangiophores with terminal, round, spore bearing sporangia. Rhizoids are absent (LPCB x400).

Other species of moulds isolated include phaeoid fungus (*Bipolaris* species (N=2) and *Curvularia* species (N=1)), *Acremonium* species (N=1) and *Fusarium* species (N=1) [Table/Fig-8].

Total	n (%)
<i>Candida albicans</i>	56 (45.9)
<i>Candida tropicalis</i>	17 (13.93)
<i>Candida parapsilosis</i>	10 (8.19)
<i>Candida glabrata</i>	9 (7.37)
<i>Candida krusei</i>	1 (0.81)
<i>Aspergillus fumigatus</i>	6 (4.91)
<i>Aspergillus flavus</i>	6 (4.91)
<i>Aspergillus niger</i>	1 (0.82)
<i>Aspergillus terreus</i>	1 (0.82)
<i>Rhizopus</i> species	5 (4.09)
<i>Mucor</i> species	4 (3.27)
Phaeoid fungus	3 (2.45)
<i>Trichosporon</i> species	1 (0.82)
<i>Acremonium</i> species	1 (0.82)
<i>Fusarium</i> species	1 (0.82)

[Table/Fig-8]: Distribution of various fungal isolates from respiratory samples.

DISCUSSION

The respiratory system is one of the primary location for fungal infections to occur, and in many instances, colonisation precedes the advancement to pulmonary fungal infection. The occurrence

of pulmonary mycosis has risen in recent decades owing to the extensive use of broad-spectrum antibiotics, immunosuppressive therapies, and chemotherapy agents, along with the growing prevalence of respiratory conditions such as Chronic Obstructive Pulmonary Disease (COPD), lung cancer, and tuberculosis [13].

Among the 560 respiratory specimens received from patients with suspected fungal respiratory tract infections during the study period, 122 samples yielded fungal growth on culture. The prevalence of respiratory mycotic infections was found to be 21.78%. A study conducted by Hassanpour P et al., showed a comparable prevalence of 31.6% in their study [14]. In contrast, Shoham S and Marwaha S reported a higher prevalence of 82.7% in their study [15]. The mean age group where fungal isolates were positive in this study was 44.91 years, which was similar to a study conducted by Chalana M et al., [11]. The relatively higher occurrence of fungal isolates in middle-aged and older individuals may be related to the increased prevalence of underlying co-morbidities such as diabetes mellitus, chronic lung diseases, malignancies, and prolonged exposure to environmental and occupational risk factors in these age groups. Age-associated alterations in immune function may also contribute to increased susceptibility to fungal colonisation and infection [16]. However, the present study did not evaluate these risk factors, and therefore no association could be established. There was a male predominance seen, 68.85% being male patients and 31.15% being female patients, consistent with the findings by Rafat Z et al., [6]. This male predominance may be due to risk factors like chronic smoking, occupational exposure, and socio-demographic conditions [17]. However, these variables were not assessed in the present study, and hence the observed male predominance cannot be attributed to these risk factors.

The current investigation showed that the most common causes of respiratory fungal infections were *Candida* species, followed by *Aspergillus* species. This finding was consistent with studies conducted by Chalana M et al., Biswas D et al., and Al-Khalidi AA et al., [11,18,19]. *Candida albicans* was the most frequently isolated species among *Candida*, followed by non-*albicans* *Candida* species such as *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata*. In respiratory mould infections, *Aspergillus fumigatus* and *Aspergillus flavus* was equally responsible. Greater environmental distribution of *Aspergillus* spores can enhance exposure thus elevating the risk of colonisation and infection by this species. In most of the studies, *Candida albicans* and *Aspergillus flavus* were specified as the most prevalent aetiology of pulmonary fungal colonisation and/or infection [6,20-23].

The higher frequency of *Candida* species compared to *Aspergillus* species observed in the present study may be attributed to the fact that *Candida* species commonly colonise the oral cavity and upper respiratory tract and are more likely to be isolated from respiratory specimens, particularly in hospitalised patients [17]. Predisposing factors such as prolonged antibiotic therapy, diabetes mellitus, corticosteroid use, intensive care unit stay, mechanical ventilation, and other immunocompromised states can promote overgrowth and colonisation by *Candida* species [17]. In contrast, *Aspergillus* species are environmental moulds acquired primarily through inhalation of airborne conidia, and their isolation is often associated with underlying structural lung diseases such as COPD, tuberculosis, bronchiectasis, and immunosuppression [24]. *Aspergillus* species were predominantly isolated from patients presenting with chronic cough, fever, and dyspnoea. The higher recovery of *Candida* species in the present study may therefore reflect the greater prevalence of these host-related risk factors among the study population. In the present study, *Candida* species were most frequently isolated from sputum samples, followed by BAL specimens. This suggests that *Candida* species were predominantly recovered from lower respiratory tract specimens, indicating that sputum was the commonest clinical sample yielding *Candida* isolates.

Systemic fungal infections are still difficult to diagnose quickly, and the sluggish growth of fungal isolates from patient specimens frequently causes delayed culture detection of fungal populations. KOH mount examination and fungal culture isolation are good diagnostic methods for the identification of fungal elements from the clinical samples [25]. Early detection of fungus by microscopic examination (KOH examination) helps early initiation of antifungal therapy, which is critical in reducing the high mortality rate. Knowing the prevalent fungal species in a given geographical area gives clinicians a rough idea about the possible causative fungal pathogens, and antifungal susceptibility report if available, it helps to start proper antifungal agent reducing the morbidity and mortality of the patients.

Limitation(s)

Because the current study was a retrospective laboratory-based investigation carried out at a single tertiary care facility, the results may not be typical of the general population and are prone to the selection bias that characterises hospital-based research. The study relied primarily on microbiological records, and detailed clinical information regarding patient co-morbidities, risk factors, treatment history, and outcomes was not available for comprehensive analysis. Moreover, the isolation of fungus, especially *Candida* species, from respiratory specimens may occasionally indicate colonisation rather than a true infection. Antifungal Susceptibility Testing (AFST) was not performed, limiting the ability to correlate the isolated fungal pathogens with their susceptibility patterns and guide targeted antifungal therapy. Future prospective multicentric studies incorporating clinical correlation, AFST, and patient outcome analysis would provide a more comprehensive understanding of respiratory fungal infections.

CONCLUSION(S)

In the present study, fungal isolates were isolated from 21.78% of respiratory specimens obtained from patients with suspected fungal respiratory tract infections. *Candida* species were the predominant isolates, with *Candida albicans* being the most frequently identified species, followed by *Aspergillus* species. However, isolation of *Candida* species from respiratory samples alone does not establish a causative role in pneumonia, as these organisms may represent colonisation of the respiratory tract. Additional clinical, radiological, histopathological, and microbiological evidence is required to differentiate colonisation from true invasive fungal infection and to establish their aetiological significance. A higher proportion of fungal isolates was observed among middle-aged adults and male patients. The findings highlight the importance of routine mycological evaluation of respiratory specimens for the early detection of fungal pathogens. Knowledge of the local distribution of fungal isolates may assist clinicians in the interpretation of laboratory findings and support appropriate patient management.

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