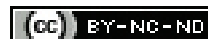


Prevalence of Lower Respiratory Tract Fungal Infection at a Tertiary Care Hospital in Central Madhya Pradesh, India: A Cross-sectional Study

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ABSTRACT

Introduction: The incidence of fungal infections and resistant isolates has risen exponentially due to multiple factors. This makes the isolation of fungi, performing identification, and susceptibility testing a necessary routine microbiological procedure. Assessing the burden and analysing the epidemiologic trends of fungal infections is critical for patient management.

Aim: To determine the prevalence of various fungal infections affecting the lower respiratory tract.

Materials and Methods: In this cross-sectional study conducted at Department of Microbiology, Netaji Subhash Chandra Bose (NSCB) Medical College Jabalpur, Madhya Pradesh, India specimens from 400 patients with suspected Lower Respiratory Tract Infection (LRTI) were processed following standard microbiological methods for fungal culture and sensitivity testing. All the fungal isolates were obtained and their antifungal susceptibility pattern were recorded. The data entry was done in a Microsoft Excel spreadsheet, and the final analysis was performed using the Statistical Package for Social Sciences (SPSS) software, version 25.0, developed by IBM in Chicago, USA.

Results: The majority of the assessed specimens were sputum (285, 71.2%). Out of the 115 *Candida* isolates, *C. albicans* (61, 53%) was the most common species isolated. Among the Non-*Candida albicans Candida* (NCAC) group, *C. tropicalis* (32, 27.8%) was the predominant isolate, followed by *C. glabrata* (16, 13.9%) and *C. krusei* (6, 5.2%). *C. albicans* was found to be the most prevalent in the elderly population (>60 years of age), whereas NCAC was prevalent in the 46-60 years age group. Regarding the antifungal drug susceptibility pattern, significantly higher drug resistance was seen in the NCAC group compared to *C. albicans* towards agents such as ketoconazole, fluconazole, itraconazole, voriconazole, amphotericin-B, and nystatin. None of the fungal isolates demonstrated resistance to caspofungin.

Conclusion: Fungal aetiology is a significant problem in patients with LRTI, which often remains underdiagnosed, increasing morbidity and mortality. Therefore, it should be kept in mind while managing a patient with LRTI.

Keywords: Antifungal drug resistance, Antifungal susceptibility test, *Candida* species, Fungemia

INTRODUCTION

Fungal infections are responsible for significant morbidity and mortality worldwide. Recently, during the ongoing COVID-19 pandemic, an upsurge in fungal infections was observed, and cases were reported from different regions of the Indian subcontinent. Fungal diseases kill more than 1.5 million and affect over a billion people annually. However, they are still a neglected entity by public health authorities, even though most deaths from fungal diseases are avoidable [1]. Among all the fungal infections, those involving the respiratory tract are largely unrecognised, and the true burden is elusive [2]. A systematic review to assess the burden of serious fungal infection in India, published in 2022, states that no concerted attempt has been made to holistically estimate the fungal burden in this country with over 1.3 billion people [3]. Also, there is no regional data on the prevalence of fungal Lower Respiratory Tract Infections (LRTI).

Candida species are unscrupulous yeasts that are known to cause infections ranging from simple infections to potentially life-threatening fungemia [4]. In recent years, NCAC species have been considered major pathogens causing severe infections in humans [5]. Quantifying the burden of fungal diseases is challenging as these infections are often difficult to diagnose because they manifest with non specific symptoms and are not routinely suspected [6]. The commonly used antifungal drugs show significant variation in the susceptibility pattern among different types of *Candida* species.

The drug resistance scenario has been increasing during the last decade due to the overuse of random antifungal agents [7]. Several previous studies have reported the emergence of drug-resistant *Candida* species in the global scenario [8,9]. The aim of the study was to determine the prevalence of various fungi affecting the lower respiratory tract. The objective was to estimate the regional prevalence of fungal LRTI and to determine their antifungal drug susceptibility pattern in patients.

MATERIALS AND METHODS

The present cross-sectional study was conducted at Department of Microbiology, NSCB Medical College Jabalpur, Madhya Pradesh, India over a period of 18 months, starting from February 2021 and continuing until August 2022. The study received approval from the Institutional Ethics Committee (IEC) with IEC approval number IEC/2020/48.

Inclusion criteria: All samples representative of the lower respiratory tract received in the microbiology laboratory for fungal culture and sensitivity were included in the study. Sputum samples were first assessed microscopically using Bartlett's grading criteria [10,11], and only acceptable samples were included in the study.

Exclusion criteria: Samples collected in an inappropriate and unsterile manner. Sputum samples with a zero or negative Q score on Bartlett's grading criteria were excluded from the study.

Sample size: The minimum sample size was calculated as 341 using the following formula [12]:

$$n = Z^2 p \cdot q / d^2$$

Therefore, a total of 400 specimens with suspected LRTI were processed.

Procedure

Specimens such as sputum, pleural fluid, and Bronchoalveolar Lavage (BAL) were collected. Demographic variables (age, sex, outpatient or inpatient category) of the patients were recorded for all the samples. Samples were processed following standard microbiological procedures for bacterial and fungal microscopy and culture to yield bacterial growth only, fungal growth only, bacterial and fungal growth, or no growth. Antifungal susceptibility testing was performed and interpreted for all *Candida* isolates using the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) M44-A document guidelines [13]. The various bacterial, fungal, or mixed growth observed were stratified with respect to age groups, gender, and outpatient/inpatient population. The distribution pattern of bacterial or fungal species isolated as a single entity or as mixed growth was recorded, and their prevalence was calculated. A structured data collection tool was used to record the result of gram stain, 10% Potassium Hydroxide (KOH) mount, bacterial and/or fungal isolates detected, and antifungal susceptibility tests.

STATISTICAL ANALYSIS

The data entry was done in a Microsoft Excel spreadsheet, and the final analysis was performed using SPSS software, version 25.0, developed by IBM in Chicago, USA.

RESULTS

In total, 400 LRTI specimens were processed. The specimens collected from inpatients and outpatients consisted of sputum in 285 (71.2%) cases, pleural fluid in 79 (19.8%) cases, and BAL in 36 (9%) cases. Among all the processed specimens, 252 (63%) were isolated from male patients and 148 (37%) were from female patients.

Out of the 115 isolated fungal agents, *C. albicans* was the most common fungi, with 61 (53%) isolates [Table/Fig-1].

Name of the isolate	n (%)
<i>Candida albicans</i>	61 (53)
<i>Candida tropicalis</i>	32 (27.8)
<i>Candida glabrata</i>	16 (13.9)
<i>Candida krusei</i>	06 (5.2)
Total	115

[Table/Fig-1]: Prevalence of fungal isolates.

C. albicans was reported to be most prevalent in the elderly population (>60 years of age), while NCAC species were most prevalent in the 46-60 years age group. No fungal isolates were found in patients up to 15 years of age [Table/Fig-2].

Fungal isolates	Upto 15 years (n=0)	16-45 years (n=23)	46-60 years (n=47)	>60 years (n=45)	Total
<i>Candida albicans</i>	0	11 (47.83%)	23 (48.94%)	27 (60%)	61 (53.04%)
Non <i>Candida Albicans</i> <i>Candida</i> (NCAC)	0	12 (52.17%)	24 (51.06%)	18 (40%)	54 (46.96%)
Total	0	23 (100%)	47 (100%)	45 (100%)	115 (100%)

[Table/Fig-2]: Distribution of fungal isolates among various age (years) groups.

Among the outpatient population, 9 (75%) of the fungal isolates were *C. albicans*, while among the inpatient population, *C. albicans* and NCAC were almost equally prevalent, with 52 (50.4%) and 51 (49.5%) isolates, respectively [Table/Fig-3].

Isolate	Outpatient (12)	Inpatient (103)	Total
<i>Candida albicans</i>	9 (75%)	52 (50.4%)	61 (53%)
NCAC	3 (25%)	51 (49.5%)	54 (47%)
Total	12	103	115

[Table/Fig-3]: Distribution of fungal isolate among inpatient and outpatient groups.

Out of the total samples, 101 (25.25%) were found to have the presence of bacterial isolates only, while 26 (6.5%) samples were positive for isolated fungal infection. Additionally, 89 (22.25%) samples were reported to be positive for the presence of mixed (bacterial and fungal) infection, and the remaining 184 (46%) samples showed no growth.

Among inpatients, isolated fungal culture was found in 21 (80.8%) cases, and mixed culture was found in 82 (92.1%) cases, significantly higher rates compared to outpatients, where isolated fungal infection was found in 5 (19.2%) cases and mixed bacterial and fungal culture in 7 (7.9%) cases [Table/Fig-4].

In/outpatient	Bacterial isolates only (n=101)	Fungal isolates only (n=26)	Fungal+Bacterial (mixed) infection (n=89)	Negative culture (n=184)	Total
Outpatient	31 (30.7%)	5 (19.2%)	7 (7.9%)	78 (42.4%)	121 (30.2%)
Inpatient	70 (69.3%)	21 (80.8%)	82 (92.1%)	106 (57.6%)	279 (69.8%)
Total	101 (100%)	26 (100%)	89 (100%)	184 (100%)	400 (100%)

[Table/Fig-4]: Bacterial and fungal culture results.

Among the 115 fungal isolates, 73 (63.5%) samples were positive for fungal isolates on 10% KOH and gram stain. Out of these, 37 (32.17%) samples showed the presence of pus cells as well as gram-positive budding yeast-like cells with pseudohyphae, and the remaining 36 (31.30%) samples showed the presence of pus cells and gram-positive yeast-like budding cells only. In the rest of the specimens, no fungal elements were observed on microscopic examination [Table/Fig-5].

Gram stain and 10% KOH mount result	Number (N=115)
Pus cells with gram positive budding yeast like cells with pseudohyphae	37 (32.2%)
Pus cells with gram positive budding yeast like cells without pseudohyphae	36 (31.3%)
No fungal element	42 (36.5%)
Total	115

[Table/Fig-5]: Microscopy results of fungal culture positive specimens.

In the present study, the antifungal drug susceptibility pattern for commonly used antifungal agents was compared between *C. albicans* (n=61) and NCAC (n=54) fungal isolates. Significantly higher drug resistance was observed in the NCAC group compared to *C. albicans*, with respect to antifungal agents, namely ketoconazole (38/54, 70.4%), fluconazole (16/54, 29.6%), itraconazole (14/54, 25.9%), amphotericin-B (11/54, 20.4%), voriconazole (6/54, 11.1%), and nystatin (10/54, 18.5%). No resistance to caspofungin was found in any of the fungal isolates [Table/Fig-6].

Anti-fungal drugs	<i>C. albicans</i> (n=61)	<i>C. glabrata</i> (n=16)	<i>C. krusei</i> (n=6)	<i>C. tropicalis</i> (n=32)
Ketoconazole	11 (18.03%)	11 (68.75%)	6 (100%)	21 (65.63%)
Fluconazole	2 (3.27%)	10 (62.50%)	Intrinsic resistance	6 (18.75%)
Itraconazole	2 (3.27%)	3 (18.75%)	4 (66.67%)	7 (21.88%)
Voriconazole	0	4 (25%)	0	2 (6.25%)
Amphotericin-B	1 (1.63%)	1 (6.25%)	2 (33.33%)	8 (25%)
Nystatin	1 (1.63%)	1 (6.25%)	4 (66.67%)	5 (15.63%)
Caspofungin	0	0	0	0

[Table/Fig-6]: Antifungal drug resistance pattern.

DISCUSSION

The respiratory tract is continuously exposed to fungal spores present in the environment, and studies have shown the presence of viable fungi at high rates in sinonasal mucus and sputum cultures, even in healthy subjects [14,15]. However, the incidence of pulmonary mycosis has increased over the past few decades due to the widespread use of broad-spectrum antibiotics, immunosuppressive and chemotherapeutic agents, as well as the increased prevalence of respiratory diseases such as Chronic Obstructive Pulmonary Disease (COPD), lung cancer, and tuberculosis [16].

In the present study, fungal and bacterial infections predominantly affected male patients. Studies have explained the male preponderance due to more prevalent associated risk factors such as smoking, alcohol consumption, and occupational exposure, which can lead to decreased local immunity in the respiratory tract, defective mucociliary clearance, mucous plugging, airway collapse, respiratory muscle fatigue, and the effects of medications used in males compared to females [17-20].

Among all the fungi isolated in this study, *C. albicans* (53%) was the predominant species. Similar prevalence figures of *C. albicans* isolation have been reported in other studies [21-23]. Among the NCAC group, *C. tropicalis* was most commonly isolated in this study, which was consistent with many published literatures [24-27]. In contrast to the findings of the present study, a study by Fleck R et al., showed a higher isolation rate of NCAC species compared to *C. albicans* [28].

In this study, *C. albicans* was reported to be most prevalent in the elderly population (>60 years of age), while NCAC was more prevalent in the 46-60 years age group. No fungal isolates were found in patients up to 15 years of age, which was similar to findings in a study by Peddapalli AR et al., [29]. It was observed that *C. albicans* was the predominant fungus in both inpatients and outpatients, which was consistent with the previous study by Rafat Z et al., [30].

The present study revealed significantly higher drug resistance in the NCAC group compared to *C. albicans*. The highest rate of drug resistance was seen with ketoconazole, which was in accordance with results seen in multiple studies [31,32]. Echinocandin resistance among *Candida* species was relatively low, with the exception being *C. glabrata*, in which echinocandin resistance is on the rise [33]. However, in the present study, none of the isolates showed resistance to caspofungin, similar to the susceptibility pattern seen in a study conducted by Ahir HR and Gohil BP, who also did not report any resistance to caspofungin. This emphasises the fact that echinocandins should be prescribed cautiously, preferably after obtaining the drug susceptibility pattern of the isolate, considering the possibility of encountering a resistant isolate [34]. This also highlights the need to closely monitor the antifungal susceptibility pattern of all *Candida* isolates by performing routine susceptibility testing.

Limitation(s)

Identification and susceptibility testing were performed using conventional methods. However, antifungal susceptibility testing using molecular tools could not be conducted due to resource constraints, which could have provided a more detailed insight into drug resistance mechanisms. Additionally, correlating the culture results of respiratory samples with blood culture could have been done to investigate invasive infections.

CONCLUSION(S)

Pulmonary fungal infection appears to be an important problem in patients with LRTI, which often goes underdiagnosed due to a lack of suspicion. This can result in increasing morbidity and mortality, especially among patients admitted to the hospital. The findings of this study support the fact that the incidence of Multidrug-Resistant (MDR) isolates are increasing, highlighting the need for strict surveillance to isolate and identify these MDR organisms.

This will allow for individually customised antifungal therapy based on susceptibility patterns and help prevent the injudicious use of antifungal agents. Further studies using molecular tools are needed to provide more specific and detailed insights into the mechanisms of antifungal drug resistance.

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