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Fungal profile of sputum samples and their Anti-Fungal Susceptibility profile in a Tertiary care Hospital in Sikkim

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Introduction: The significance of fungal growth in sputum culture remains uncertain and is typically not addressed when detected. This study aims to outline the clinical observations of individuals exhibiting fungal growth in their sputum, as well as assess their susceptibility to antifungal agents. The primary objective is to acquire an understanding of the local epidemiology related to resistance against antifungal agents. **Material and method:** This is a hospital-based, cross-sectional study conducted for one year January-December 2022 in a Tertiary care hospital in Sikkim. Sputum samples from in-patients (ICU/Ward) were processed for fungal culture and the identification was done by Chrome Candida differential agar and the automated Microbiology System for yeast and yeast-like organisms, the moulds were identified morphologically. Anti-fungal susceptibility testing (AFST) was done using antifungal disks Amphotericin B 20 mcg, Itraconazole 10 mcg, Fluconazole 25mcg and Voriconazole 1 mcg and following Standard operating procedure (SOP) manual of ICMR 2nd edition 2019. **Results:** Out of 190 samples, 23(12%) and 21 (11%) Candida spp. and moulds were isolated. Among Candida spp., Candida albicans was isolated in 20, Candida tropicalis in 2, and Candida. glabrata in 1. AFST showed 91% of Candida spp. were sensitive to Amphotericin B followed by Fluconazole (39%) and Voriconazole (35%). Among 21 moulds identified A. fumigatus (6), A. flavus (7), A. niger (1), Penicillium spp. (4), Acremonium spp. (2) and Fusarium spp. (1) were isolated. AFST showed that Voriconazole was the most sensitive (85%) followed by Amphotericin B (57%) and the most resistant antifungal was Fluconazole (71%) followed by Itraconazole (47%) in moulds. **Conclusion:** The study brought attention to prevalent fungal commensals within the respiratory tract and provided insights into the susceptibility patterns of fungal pathogens in the region.

Keywords: Fungal commensals, Sputum, Antifungal susceptibility pattern, Candida spp., Aspergillus spp

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Introduction

Determining a fungal pathogen through isolation in a sputum culture should not be confidently regarded as the primary cause of a respiratory infection unless we have eliminated the possibility of contamination from oropharyngeal flora. Invasive Fungal Diseases (IFDs) pose an increasing risk to individuals with weakened immune systems, underscoring the need for vigilant fungal pathogen surveillance. It is noteworthy that certain fungi previously considered non-pathogenic to humans exhibit virulence factors akin to their pathogenic counterparts. In this context, we draw attention to the emergence of environmental saprophytic fungi, such as Aspergillus, Penicillium, and Candida, as novel human pathogens. In certain instances, newly emerging pathogens exhibit resistance to existing antifungal medications, thereby increasing the risk associated with novel fungal diseases. [1]

Historically, treatment was constrained to amphotericin B, flucytosine, and a small selection of clinically accessible azole agents. In contemporary pharmacological strategies, there are now powerful new azole compounds with prolonged antifungal efficacy, lipid-based formulations of amphotericin B, and more recent antifungal medications, such as echinocandins, available for use. Given the changing treatment of pulmonary fungal infections, there is a need to understand the antifungal resistance pattern of pulmonary mycosis. [2] Since as much as 30%–50% of antifungal prescriptions could be optimized or are inappropriate. [3]

Multidrug-resistant Candida auris infections have been documented on five different continents in recent years. The rapid transmission of this organism among patients and from the environment to patients is a concerning factor. Hence, the primary objective of this study is to offer valuable insights into fungal infections occurring within a hospital environment. These findings will serve as crucial guidance for the future management of patients facing similar issues in this region.

Materials and Methods

Patients with respiratory symptoms from the Wards/ICUs from January to December 2022 in a Government tertiary care hospital were included in the study.

Sputum quality was checked by using Bartlett criteria and was processed following conventional mycological procedures like direct microscopy (KOH) and culture on Sabouraud dextrose agar (SDA).

Identification of yeast: For the yeasts, identification was done by morphological characteristics. For Candida spp, Candida Differential Agar and the BD Phoenix TM Automated Microbiology System for yeast and yeast-like organisms were used.

Identification of Mould: Moulds were identified by morphological characteristics, LPCB Tease mount, and Slide culture technique.

Anti-fungal susceptibility testing: Anti-fungal susceptibility testing (AFST) for yeasts and moulds was done in Mueller-Hinton Agar + 2% Glucose and 0.5 μg/mL Methylene Blue Dye (GMB) Medium. Antifungal disks used were Amphotericin B (AMB) 20 mcg, Itraconazole (ICZ) 10 mcg, Fluconazole (FCZ) 25mcg and Voriconazole (VCZ) 1 mcg. Zone interpretation criteria for yeasts and moulds have been referred from the Standard operating procedure manual of ICMR 2nd edition 2019.[3] Zone diameter epidemiological cutoff values Clinical breakpoints have not been established for mould testing, tentative epidemiological cutoff values (ECVs) are available to detect moulds with reduced susceptibility (non–wild type) to amphotericin B, caspofungin, itraconazole, posaconazole, and voriconazole.[3] The following tentative zone diameter categories were assigned for sensitive, ≥17 mm (triazoles) and ≥15 mm (amphotericin B); intermediate, 14 to 16 mm (triazoles) and 10 to 13 mm (amphotericin B); resistant, ≤10 mm (triazoles) and ≤10 mm (amphotericin B).[8] Patient's clinical history and management was analyzed and correlated retrospectively from the patient's records.

Statistical analysis was done using Microsoft Excel sheet/ Statistical Package for the Social (SPSS) version 20.0.

Results

Demographic result: A total of 190 sputum were processed, with 44 (23%) yielding fungal isolates. (Fig. No. 1) Among these isolates, 23 were identified as Candida spp. while 21 were categorized as moulds.

Notably, the age group most impacted were the patients in 55-74 years age group (7%) and the male population 34(18%) exhibited a higher susceptibility compared to the female population 10(5%). Yeast was seen most in 75-94 yrs. age group and males were affected mostly i.e., 10(83%). (Table No.1)

Table no. 1 Demographic Distribution of Fungus-Isolated from Sputum Samples (n-44) from a Population of 190 Individuals: January-December 2022 at a Tertiary Care hospital in Sikkim.

AGE GROUP (years)	MALE n (%)	FEMALE n (%)	TOTAL n (%)	YEAST		MOULD	
				MALE	FEMALE	MALE	FEMALE
15-34	7 (4%)	4 (2%)	11 (6%)	2(18%)	2(18%)	5(45%)	2(18%)
35-54	5 (2%)	2 (1%)	7 (4%)	1(20%)	0	4(57%)	2(40%)
55-74	10 (5%)	4 (2%)	14 (7%)	6(43%)	2(14%)	4(28%)	2(14%)
75-94	12 (6%)	0	12 (6%)	10(83%)	0	2(17%)	0
TOTAL	34(18%)	10(5%)	44 (23%)	19(43%)	4(9%)	15(34%)	6(14%)

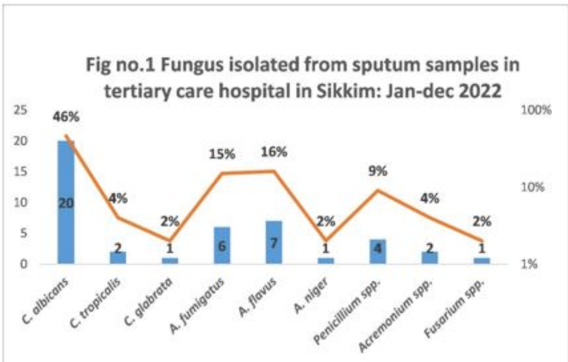

Fig no.1 Fungus isolated from sputum samples in tertiary care hospital in Sikkim: Jan-dec 2022

Clinical profile: The predominant symptoms noted were fever (88%), followed by dyspnoea (63%). Among the patients, COPD was diagnosed in 38%, while 20% were diagnosed with pneumonia. Approximately 47% of patients were receiving steroid treatment in forms (injectable or inhalational). Merely 2 patients tested positive for COVID-19 through RT-PCR, while 7 patients (30%) exhibited radiological features such as consolidation and emphysematous changes. Furthermore, 4 patients (17%) presented with co-morbidities including Tuberculosis and Diabetes mellitus.

Antifungal susceptibility testing (AFST) of Candida spp.

Out of the 23 samples, Candida albicans were isolated in 20, Candida tropicalis in 2, and C. glabrata in 1. Antifungal susceptibility testing (AFST) revealed that the Candida spp. were most sensitive to Amphotericin B (87%), followed by Voriconazole (43%) and Fluconazole (30%). They were most resistant to Itraconazole 74%. Species-specific AFST showed that Candida albicans was 85% resistant to Itraconazole, but was 100% sensitive to C. glabrata & C. tropicalis.

Antifungal susceptibility testing (AFST) of moulds

Among 21 moulds A.fumigatus 6(28%), A. niger 1(4%), Penicillium spp.4(8%), Acremonium spp.2(4%) and Fusarium spp.1(4%) were isolated. For all moulds, the AFST indicated that Voriconazole exhibited the highest sensitivity (90%), followed by Amphotericin B (57%), Fluconazole (43%) and Itraconazole (38%). Among Aspergillus spp. also the same sensitivity pattern was observed, with Voriconazole being the most sensitive (90%) followed by Amphotericin B (59%). Species-specific AFST shows that A. flavus and A. fumigatus together were 61% resistant to Amphotericin B. Fluconazole showed 100% resistant/intermediate susceptibility to Aspergillus spp. Other moulds like Acremonium spp., Fusarium spp. and Penicillium spp. were sensitive to all antifungals (>95%). (Table no. 2)

Table no. 2 Anti-Fungal Susceptibility Profile in sputum samples: Tertiary care Hospital in Sikkim: Jan-Dec 2023

	Voriconazole (VCZ) 1 mcg		Itraconazole (ICZ) 10 mcg			Fluconazole (FCZ) 25mcg			Amphotericin B (AMB) 20 mcg	
Yeast (n)	Sensitiven (%)	Resistantn (%)	Sensitiven (%)	Resistantn (%)	Intermediate n (%)	Sensitiven (%)	Resistantn (%)	Intermediate n (%)	Sensitive n (%)	Resistantn (%)
C. albicans (20)	7(35 %)	13(65 %)	0	17(85 %)	3(15%)	6(30 %)	14(70 %)	0	17(85 %)	3(15 %)
C. glabrata (1)	1(100 %)	0	1(100 %)	0	0	0	1(100 %)	0	1(100 %)	0
C. tropicalis (2)	2(100 %)	0	2(100 %)	0	0	1(50 %)	1(50 %)	0	2(100 %)	0
Total Yeast (n=23)	10(43 %)	13(57 %)	3(13 %)	17(74 %)	3(13%)	7(30 %)	16(70 %)	0	20(87 %)	3(13 %)
Mould(n)	Sensitive n (%)	Resistant n (%)	Sensitive n (%)	Resistant n (%)	Intermediate n (%)	Sensitive n (%)	Resistant n (%)	Intermediate n (%)	Sensitive n (%)	Resistant n (%)
A. niger (1)	1(100 %)	0	0	1(100 %)	0	0	1(100 %)	0	1(100 %)	0
A. flavus (7)	5(71 %)	2(28 %)	6(86 %)	1(14 %)	0	0	0	7(100 %)	3(43 %)	4(57 %)
A. fumigatus (6)	6(100 %)	0	1(17 %)	0	5(83%)	0	5(83 %)	1(17%)	2(34 %)	4(66 %)
Acremonium spp. (2)	2(100 %)	0	0	0	2(100 %)	1(50 %)	0	1(50%)	2(100 %)	0
Fusarium spp. (1)	1(100 %)	0	1(100 %)	0	0	1(100 %)	0	0	1(100 %)	0
Penicillium spp. (4)	4(100 %)	0	0	2(50 %)	2(50%)	0	3(75 %)	1(15%)	3(75 %)	1(15 %)
Total mould (n=21)	19(90 %)	2(10 %)	8(38 %)	4(19 %)	9(19%)	2(43 %)	9(19 %)	10(48 %)	12(57 %)	9(19 %)

Discussion

Demographics of this study

The demographics of this study show that the most affected age group was 55-74 years age group (7%) and the male population 34(18%) exhibited a higher susceptibility compared to the female population 10(5%). A study carried out in Guilan province, located in Iran's northern region also observed most isolates were from male patients 86 (62.77%) and most of them were between 46 and 72 years. [4]

Clinical profile

The predominant symptoms noted were fever (88%), followed by dyspnoea (63%). Among the patients, COPD was diagnosed in 38%. Furthermore, 17% presented with co-morbidities including Tuberculosis and Diabetes mellitus. The study observed a comparable situation in the study [4], where prominent symptoms included cough (94.16%), dyspnea (81.02%), purulent sputum (62.04%), and weight loss (56.2%).

The prevailing underlying conditions were tuberculosis (24.81%), chemotherapy (21.89%), and diabetes mellitus (19.70%). [4] The recent onset of Coronavirus Disease (COVID-19) has been linked to reports of fungal infections, notably aspergillosis and mucormycosis, particularly in critically ill patients who have received steroid treatments. This study revealed a clear connection between patients undergoing diverse forms of steroid treatment (such as injectable or inhalational) and the isolation of fungi.

Candida spp. and moulds isolated

This study revealed the Candida sp. isolated was 12% and almost the same percentage of moulds were isolated (11%).

In a study done in the Himalayan region of India, among 28 patients Candida and Aspergillus were recovered from 14 and 13 patients respectively.[5], Out of 23 Candida spp. isolated, 20 Candida albicans were isolated, Candida tropicalis 2, and C. glabrata 1. Among 21 moulds A. Fumigatus 6(28%), A. niger1(4%), Penicillium spp.4(8%), Acremonium spp.2(4%) and Fusarium spp.1(4%) were isolated. Similar isolated species were seen in the study in the Himalayan region.[5]

In a tertiary referral Hospital, in northeast India patients were examined for pulmonary mycoses and discovered Pulmonary candidiasis in 50% of the patients where Candida albicans showed the highest incidence of association, followed by five other Candida species. [6]

Candida spp. and their Antifungal susceptibility testing (AFST)

Antifungal susceptibility testing (AFST) revealed that the Candida spp. were most sensitive to Amphotericin B (87%), followed by Voriconazole (43%) and Fluconazole (30%). In a cross-sectional study in Lebanon, clinical samples were collected from 258 pregnant women with vaginal discharge. The observed susceptibility rates of C. albicans isolates to Amphotericin B, Fluconazole, Itraconazole and Voriconazole were 97.5, 90, 87.5 and 97.5%, respectively. [7]

The total resistance rates of itraconazole (23%) were highest in a study in China followed by Voriconazole (18.5%) respectively.[8]

This confers to the present study, where Itraconazole is most resistant (74%) and Amphotericin B, Fluconazole and Voriconazole are more sensitive to candida spp.

It was concluded in a study that the magnitude of the potential adaptive responses that C. albicans is capable of and these responses may have a significant impact on its susceptibility to AMB. Given that the range of environments capable of triggering these adaptive responses is likely much broader in vivo than in vitro, it's understandable that conducting antifungal susceptibility studies in patients can become quite intricate.[9]

Mould and their Antifungal Susceptibility Testing (AFST)

The AFST in moulds indicated that Voriconazole exhibited the highest sensitivity (90%), followed by Amphotericin B (57%), Fluconazole (43%), and Itraconazole (38%). Aspergillus spp. primarily impacts immunosuppressed patients, leading to higher mortality rates. Voriconazole is the preferred treatment for this condition. However, the concerning rise in the number of azole-resistant isolates in recent years has garnered significant attention, as it directly correlates with an uptick in clinical treatment failures.[10]

There are three primary categories of contemporary antifungal drugs employed in the management of mould infections: the triazole antifungals (such as posaconazole and voriconazole), the echinocandins (including caspofungin, micafungin, and anidulafungin), and therapy based on amphotericin B (AMB). Each of these drug classes has its limitations when it comes to their effectiveness against various types of mould. For example, voriconazole has excellent activity against Aspergillus species [11] and modest activity against Fusarium species.[12] In a study in India, they proposed the possible role of multidrug efflux pumps, in contributing to azole resistance in *A. flavus*.[13] Although in the present study, Voriconazole is showing high sensitivity (90%) in moulds, close supervision is needed.

Conclusion

Despite inherent challenges, optimism remains strong in the current situation of antifungal therapeutics. Although sputum is considered not a very good sample, the Institute doesn't have a facility for conducting Broncho alveolar lavage (BAL) samples, considering a resource-limited facility the sputum samples were studied in the study.

There are distinct patterns of geographical variation in fungal infections and it is essential to determine the local etiology within a given region when planning a management strategy. In this study, the local epidemiology is similar to the rest of the country. This study also has a limitation because there is no evidence to show that the isolates are pathogenic or commensal microbiota within the respiratory tract. This research offers valuable reference data that can be used in future epidemiological and susceptibility studies concerning antifungal agents in the region.

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During the preparation of this work, the author(s) used [Chat GPT 3.5] to [To improve language]. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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