Prevalence of Fungal Infections Among Patients with Chronic Lung Diseases in a Tertiary Care Hospital: A Cross-Sectional Study

Akash K. Dumbhare[1], Nirmal Channe[2], Sunita Gajbhiye[3], Sunanda Shrikhande[4]

[1]Department of Medical Microbiology,
Government Medical College and Hospital, Nagpur, Maharashtra , India; 440003

Abstract: Introduction: Fungal infections are becoming a critical global public health concern due to the rise in immunocompromised persons, as well as the number of people who are terminally ill or incapacitated and frequently use broad-spectrum antibiotics. Infections with fungus are commonly observed in people with underlying chronic lung diseases. The current study aims to investigate prevalence of fungal infections in the respiratory tract in CLD cases. Methods: Clinical specimens, including sputum, induced sputum, and broncho-alveolar lavage, were collected aseptically. Yeast and mold cultures were identified based on cultural and morphological characters. Then, antifungal susceptibility was checked using the disc diffusion method. Antifungal susceptibility testing (AFST) provides an in vitro measure of susceptibility and resistance by determining the concentration of drug required to inhibit the yeast and mold cultures to a specified degree, termed the MIC. The MIC values can be used to guide patient therapy, inform epidemiological studies, and track rates of antifungal drug resistance [37]. Results: Only Candida albicans and Candida dublinensis showed positive results for the germ tube test. Of the 197 specimens, 118 showed fungal growth, whereas 79 had no growth. Out of the positive individuals, 83 (42.1%) belonged to the age group of 41-50 years, followed by 42 (21.4%) in the age group of 31-40 years, 38 (19.3%) in the age group of 51-60 years and only seven cases (3.6%) were reported in the age group above 60 years of age and three patients were below ten years of age. Conclusion: Rapid diagnosis and treatment are essential for improving outcomes and reducing mortality. More research is needed to develop innovative diagnostic methods, treatments, and effective prevention strategies.

Keywords: CLD , Candida albicans, Candida dublinensis, germ tube test

INTRODUCTION

A substantial number of lives are lost due to fungal infections, resulting in a significant death toll, with over 1.5 million fatalities annually [1]. A growing number of people with immunological weaknesses, as well as terminally sick and disabled individuals who require frequent use of broad-spectrum antibiotics, are making fungal infections an increasingly severe global public health concern [15]. Fungi infections are frequently seen in patients with underlying chronic pulmonary illnesses [3, 17]. The key factors contributing to fungal infections in chronic lung illnesses are increased mucus secretion and airway inflammation [4, 36]. Smoking or using biomass fuel can compromise the mucociliary clearance function, weakening the body's defense against respiratory diseases [5]. Aspergillus, Candida, Cryptococcus species, Pneumocystis jiroveci, and Mucor-mycosis continue to be the most prevalent pathogens of fungi causing a wide range of severe fungal diseases, with dietary depletion, particularly undernutrition, serving as another significant contributing factor [6]. This is true even though the epidemiology of fungal diseases has significantly changed over the past few decades [2]. The most common human fungi pathogens include Aspergillus spp., Cryptococcus spp., and Pneumocystis spp., which cause airborne opportunistic fungal infections. Similarly, Candida albicans, a polymorphic commensal fungus species associated with humans, is the most typical pathogen discovered in the fungal respiratory mycoses [7]. More than 90% of deaths from fungal infections are caused by these fungi [8].

The use of antifungal medication is debatable, solely dependent on clinical grounds, and predicated on the premise that the species isolated from culture media is pathogenic, making fungal infections challenging to treat [9]. Resistance to drugs remains a significant issue that restricts the use of antifungal medications [10].

Chronic lung illnesses, which include a combination of COPD, asthma, restrictive lung diseases, interstitial lung diseases, and chronic respiratory infections, predispose the patient to a variety of respiratory fungal infections [12, 13]. Though the causes of fungal infection of the pulmonary system in cases of CLD are still unknown, some of the hypothesized causes include the function of numerous cells and molecules that coordinate the body's reaction to an infection caused by fungi in the lung. Innate myeloid cells, such as neutrophils, macrophages, and dendritic cells (DC), act as the first line of defense when a fungal infection threatens the chronically harmed lungs. These cells intend to

prevent infection and attack the invading pathogens. The cells responsible for triggering the phagocytosis of pathogens such as pathogenic bacteria, fungi, and malignant cells also release immunomodulatory cytokines to combat infection [38] Cytokines released by these innate immune cells play critical roles in regulating the immune response, including the modulation of antigen-presenting cells (APCs) and effector lymphocytes [39]. These cytokines serve as intercellular messengers, providing soluble regulatory signals that both initiate and constrain inflammatory responses to pathogens and injury [40].Later on, recruited natural killer cells kill invaders directly and indirectly to regulate the growth of fungi in the lungs. The development of fungal respiratory mycosis occurs when these systems are compromised, either due to persistent lung involvement in CLD or related immune deficient diseases [14]. The current investigation must thoroughly define the characteristics of fungal infections in the respiratory tract in instances of CLD due to the lack of literature on the potential fungi affecting individuals with chronic lung illnesses.

MATERIAL AND METHODS

Sample collection. Clinical specimens, including sputum, induced sputum, and broncho-alveolar lavage, were collected aseptically in clean, sterile containers, adequately sealed and labeled, and sent to the laboratory.

Microscopic Examination:

Potassium hydroxide wet mount (KOH Mount). A small amount of specimen was mixed with a drop of 10% KOH on a clean, grease-free glass slide. A sterile cover slip was placed on the mixture, and kept in a moist chamber at room temperature for 10 min, allowing debris to clear. The sample was then observed under low (10X) and high power (40X) of the light microscope to check for the presence of yeasts or hyphal forms.

Yeast and yeast-like fungi:

Culturing and identification. The samples were inoculated aseptically in two sets of Sabouraud's Dextrose Agar (SDA with antibiotics) chloramphenicol and gentamicin 50 mg/liter. These samples were incubated at 25 ^{0}C and 37 ^{0}C until growth was obtained for about 3-4 weeks. Sputum samples were inoculated on Sabouraud's Dextrose Agar to observe fungal growth.

When growth was observed, the colony morphology was noted, and India ink preparation was made. The isolate was further processed for species identification based on microscopic and colony morphology.

Gram staining and capsule staining were carried out for the identification of cultures. A carbohydrate fermentation test was also performed using different Sugars like sucrose, maltose, and lactose.

Molds:

Culturing and identification. The culturing procedure and the media (SDA) used for both, molds as well a yeasts were the same. Colonies were observed on SDA, and morphology was noted. On the basis of morphological characteristics, genus level identification was done.

Lactophenol cotton blue (LCB) tease mount. Lactophenol Blue Solution is a mounting medium and staining agent used to prepare slides for microscopic examination of molds. The colony fragment was placed on a microscope slide in a drop of lactophenol aniline blue, and the colony was teased apart with the dissecting needles and overlayed with a cover slip. The preparation was examined under the microscope under 10X and 40X.

In vitro antifungal susceptibility by disc diffusion method. The strains of candida species (n=65) isolated from the samples, were subjected to susceptibility testing against fluconazole and voriconazole by disc diffusion as per CLSI guidelines M44-A2.

Inoculum was prepared by picking up colonies from 24-hour-old candida species culture and suspending in 5 ml of sterile 0.145 mol/L saline (8.5 g/L NaCl; 0.85% saline). The resulting suspension was vortexed for 15 seconds, and its turbidity was adjusted visually by adding sufficient sterile saline or more colonies to adjust the transmittance to that produced by a 0.5 McFarland standard at 530 nm wavelength.

The steroid Mueller-Hinton + GMB agar plate was evenly streaked with this culture suspension using a swab. As a final step, the rim of the Agar was swabbed. Antimicrobial disks were placed on an inoculated Agar plate, and the plates were incubated at 35 ^{0}C ($\pm 2^0$ C) for 20 – 24 hours.

RESULTS

A. Identification of yeasts

a) Candida species

1. Colony morphology: Various Candida species showed different morphological characteristics on SDA (Table 1).

Table 1. Colony morphology of Candida species

Species	Morphology
C. albicans	Creamy, soft, pasty
C. tropicalis	Cream-colored to off-white, glistening to dull, soft, smooth, or wrinkled
C. glabrata	Glistening, smooth, cream-coloured
C. parapsilosis	Creamy with lacy pattern 60
C. kursei	Creamy dry after prolonged incubation, yellowish, dull, and wrinkled
C. kefyr	Creamy smooth become cream to yellow
C. guilliermondii	Flat, glossy, cream to pink, dull

2. Germ tube test:

Only Candida albicans and Candida dublinensis showed positive results for the germ tube test.

3. CHROM agar Candida medium (biochemical test):

Table 2. Colony morphology on CHROM agar Candida medium

Species	Colony colour
C. albicans	Light green
C. tropicalis	Blue with a pink halo
C. parapsilosis	Cream to pale pink
C. glabrata	Pink to purple
C. kursei	Pale pink colonies
C. lusitaniae	Pink gray purple
C. guilliermondii	Pink to purple colonies

4. Carbohydrate fermentation test:

Table 3. Carbohydrate fermentation of Candida species

Candida Species	Glucose	Maltose	Sucrose	Lactose
C. albicans	Acid and gas	Acid and gas	-	-
C. tropicalis	Acid and gas	Acid and gas	Acid and gas	-
C. parapsilosis	Acid and gas	-	-	-
C. glabrata	Acid and gas	-	-	-

b) identification of Cryptococcus spp.:

Microscopic morphology showing gram-positive, round to oval yeast cells with single narrow-based budding denoted Cryptococcus.

B. Identification of Molds:

Table 4. Colony morphology of Aspergillus species

Species	Phialides	Colour of colony
A. fumigatus	Uniseriate	Velvety, powdery at first, turning to smoky green. Reverse white to tan
A. flavus	Biseriate/Uniseriate	Velvety, yellow to green or brown Reverse golden to red-brown
A. niger	Biseriate	Woolly at first white to yellow, then turning dark brown to black, Reverse white to yellow.
A. terreus	Biseriate	Cinnamon brown, Reverse white to brown
A. nidulans	Biseriate	Dark green, Reverse olive to drab gray or purple-brown

Table 5. Distribution of cases according to Age (n=197)

Age (in years)	Number	Percentage
< 10 years	3	1.5
10-20	5	2.5
21-30	19	9.6
31-40	42	21.4
41-50	83	42.1
51-60	38	19.3
>60	7	3.6
Total	**197**	**100**
Mean ± SD (years)	**54.6 ± 4.7**	

Table 6. Gender-wise distribution of cases (n=197)

Gender	Number	Percentage
Male	126	63.9
Female	71	36.1
Total	**197**	**100**

Table 7. Ward-wise distribution of cases (n=197)

Admitted wards	Number	(%)
COVID ward	84	42.7
Respiratory medicine ward	76	41.7
SARI wards	30	15.2
Medicine wards	4	2
Paediatrics wards	3	1.5
Total	**197**	**100**

Table 8. Distribution of cases according to co-morbid conditions (n=197)

Co-morbid conditions	Number	(%)
Diabetes Mellitus	62	31.5
Asthama	47	23.9
COPD	21	10.7
Hypertension	4	2
IHD	7	3.6
Chronic Renal Disease	4	2
NO associated Co-morbid conditions	52	26.4
Total	**197**	**100**

Table 9. Distribution of the clinical specimens collected (n=197)

Specimen	**Number**	**(%)**
Sputum	163	82.7
Broncho-alveolar Lavage	34	17.3
Total	**197**	**100**

Table 10. Distribution of fungal isolates in clinical specimens (n=197)

Specimen	**Growth No (%)**	**No Growth No (%)**	**Total**
Sputum	87 (73.8)	76 (96.2)	163 (82.7)
Broncho-alveolar Lavage	31 (26.2)	3 (3.8)	34 (17.3)
Total	**118 (59.89)**	**79(40.10)**	**197 (100)**

Table 11. Distribution of fungal isolates (n=118)

Fungal isolates	**Number obtained (%)**
Aspergillus spp.	69 (58.4)
Candida spp.	23 (19.4)
Mucor spp.	21 (17.7)
Cryptococcus spp.	5 (4.2)
Total	**118 (100)**

Table 12. Distribution of fungal isolates according to clinical specimens (n=118)

Specimen	**Aspergillus spp. (%)**	**Candida spp. (%)**	**Mucor spp. (%)**	**Cryptococcus spp. (%)**	**Total No (%)**
Sptutum	49 (41.5)	16 (13.6)	19 (16.1)	3 (2.5)	87 (73.7)
Broncho-alveolar Lavage	20 (16.9)	7 (5.9)	2 (1.7)	2 (1.7)	31 (26.3)
Total No (%)	**69 (58.4)**	**23 (19.4)**	**21 (17.7)**	**5 (4.2)**	**118 (100)**

Table 13. Species-wise distribution of fungal isolates (n=118)

Fungal isolates	**Number (%)**
Aspergillus spp. (n=69)	
A. fumigatus	37 (31.3)
flavus	23 (19.5)
ger	9 (7.6)
Candida spp. (n=23)	
C. albicans	15 (12.8)
C. tropicalis	5 (2.5)
C. glabrata	3 (17.8)
Mucor spp. n=21 (17.8)	
Cryptococcus spp. n=5 (4.2)	
Total	**188 (100)**

Table 14. Distribution of fungal isolates according to age (n=118)

Age (Years)	**Aspergillus spp. (%)**	**Candida spp. (%)**	**Mucor spp. (%)**	**Cryptococcus spp. (%)**	**Total No (%)**
< 10 years	2	0	0	0	2 (1.6)
10-20	3	3	1	0	7 (5.9)
21-30	7	0	1	0	8 (7.0)
31-40	7	4	6	0	17 (14.4)
41-50	24	13	8	0	45 (38.1)
51-60	21	2	5	3	31 (26.2)
>60	5	1	0	2	8 (6.7)
Total (%)	**69 (58.4)**	**23 (19.4)**	**21 (17.8)**	**5 (4.2)**	**118 (100)**

Table 15. Distribution of fungal isolates according to gender (n=118)

Gender	Aspergillus spp. (%)	Candida spp. (%)	Mucor spp. (%)	Cryptococcus spp. (%)	Total No (%)
Male	42	10	14	2	**68(57.6)**
Female	27	13	7	3	**50(42.4)**
Total (%)	**69 (58.4)**	**23(19.4)**	**21(17.8)**	**5(4.2)**	**118(100)**

Table 16. Distribution of fungal isolates according to co-morbidities (n=118)

Co-morbidities	A. fumigatus	A. flavus	A. niger	Mucor Spp.	C. albicans	Candida non albicans	Cryptococcus spp.	Total No (%)
Diabetes Mellitus (62)	14	5	2	13	8	5	5	52 (44)
Asthma (47)	11	9	2	3	4	1	0	30 (25.4)
COPD (21)	8	3	1	2	0	1	0	15 (12.7)
Hypertension (4)	1	0	1	0	0	0	0	2 (1.6)
IHD (7)	1	0	1	0	3	1	0	6 (5.0)
Chronic renal Disease (4)	0	2	1	1	0	0	0	4 (3.3)
No associated Co-morbid conditions (52)	2	4	1	2	0	0	0	9 (7.6)
Total (%)	**37 (31.4)**	**23 (19.5)**	**9 (7.6)**	**21 (17.8)**	**15 (12.8)**	**8 (6.8)**	**5 (4.2)**	**118 (100)**

Table 17. Antifungal resistant pattern of candida spp. using Fluconazole and voriconazole disc

SPECIES (n=23)	Fluconazole(25ug)	Voriconazole (1 ug)
C. albicans (15)	9 (60%)	7 (47%)
C. tropicalis (5)	2 (40%)	1 (20%)
C. glabrata (3)	3 (100%)	3 (100%)

DISCUSSION

Among the 197 specimens analyzed, 118 exhibited fungal growth, while 79 showed no growth. The majority of cases (42.1%) fell within the 41-50 age group, comprising 83 specimens, followed by 31-40 age group with 42 specimens (21.4%), and 51-60 age group with 38 specimens (19.3%). Only seven cases (3.6%) were reported in the age group above 60, and three patients were below ten years of age. The age range varied from 12 to 76 years, with a mean age of 54.6 years and a standard deviation of 4.7 years.

Studies by [18] and [19] reported mean ages of 59 years and 59.2 years, respectively, with varying ranges. The increased incidence of fungal infections with age, particularly among those above 50, may be attributed to underlying lung diseases developed over decades. Additionally, age-related declines in lung capacity and immune response may contribute to the acquisition of fungal pathologies in older individuals.

Male predominance was observed, with 126 (63.9%) males and 71 (36.1%) females. [19] also reported a male preponderance, with males constituting 62.8% of the study population. This could be attributed to outdoor occupations, increased exposure to air pollution, higher prevalence of smoking in men, and resulting underlying lung conditions predominantly seen in males.

The majority of cases were from COVID wards (42.7%), followed by Respiratory Medicine wards (41.7%), and SARI ward (15.2%). Fewer specimens were from Medicine wards (2%) and Pediatrics ward (1.5%).

Invasive fungal infections were more prevalent in patients with diabetes mellitus (31.5%), followed by asthma (23.9%) and COPD (10.7%). Hypertension and chronic renal disease were less common comorbidities.

Out of 197 specimens, 163 (82.7%) were sputum specimens and 34 (17.3%) were BAL specimens. Growth was observed in 118 specimens, with 87 (73.8%) being sputum specimens and 31 (26.2%) BAL specimens.

Aspergillus spp. accounted for the majority of fungal isolates (58.4%), followed by Candida spp. (19.4%) and Mucor spp. (17.7%). Cryptococcus spp. were less common (4.2%).

Antifungal susceptibility testing was conducted using the disc diffusion method in accordance with CLSI guidelines. Notable resistance patterns were observed among Candida species, particularly to fluconazole and voriconazole.

Specifically, resistance rates were observed in Candida albicans, with 60% resistant to fluconazole and 47% resistant to voriconazole. Among Candida tropicalis isolates, 40% showed resistance to fluconazole, with 20% resistant to voriconazole. All Candida glabrata isolates were found to be resistant to both fluconazole and voriconazole.

These findings are consistent with trends reported in previous studies. For instance, a study by [35] noted an increasing trend of fluconazole resistance in both Candida albicans and non-albicans species. They reported low incidence rates of fluconazole resistance in C. albicans (approximately 0.5–2%), but higher rates in C. tropicalis (4–9%) and C. glabrata (11–13%).

Conclusion

According to the results of the current study, pulmonary mycoses are becoming more common in both immunocompetent and immunocompromised patients in our nation. As a result, if a patient with a chronic lung infection is not improving while receiving routine antibiotic therapy, it is important to check them for fungal infection since the presence of fungal species in respiratory tract samples should not be mistaken for colonisation. A doctor should start anti-fungal medication right away if there is any sign of fungal mycoses, such as a positive sputum culture or serological test. To enhance outcomes and lower death rates, rapid diagnosis and treatment are crucial. To create novel diagnostic techniques, therapies, and successful prevention plans, further study is required.

Acknowledgement
This work was carried out at the Department of Medical Microbiology, Government medical college and Hospital, Nagpur, MS, India; 440003
Financial support and sponsorship: Nil

Conflict of interest: None to Declare

Ethical approval: Not needed

REFERENCES

1. Kainz, K., Bauer, M. A., Madeo, F., & Carmona-Gutierrez, D. (2020). Fungal infections in humans: the silent crisis. Microbial Cell, 7(6), 143.
2. Bongomin, F., Gago, S., Oladele, R. O., & Denning, D. W. (2017). Global and multi-national prevalence of fungal diseases—estimate precision. Journal of Fungi, 3(4), 57.
3. Rautemaa-Richardson, R., & Richardson, M. D. (2017). Systemic fungal infections. Medicine, 45(12), 757-762.
4. Guo, M. Y., Chen, H. K., Ying, H. Z., Qiu, F. S., & Wu, J. Q. (2021). The role of respiratory flora in the pathogenesis of chronic respiratory diseases. BioMed Research International, 2021.
5. Sonwani, S., Madaan, S., Arora, J., Suryanarayan, S., Rangra, D., Mongia, N., & Saxena, P. (2021). Inhalation exposure to atmospheric nanoparticles and its associated impacts on human health: a review. Frontiers in Sustainable Cities, 3, 690444.
6. Casadevall, A. (2022). Immunity to invasive fungal diseases. Annual Review of Immunology, 40, 121-141.
7. Pfaller, M. A., & Diekema, D. J. (2004). Rare and emerging opportunistic fungal pathogens: concern for resistance beyond Candida albicans and Aspergillus fumigatus. Journal of Clinical Microbiology, 42(10), 4419-4431.
8. Brown, G. D., Denning, D. W., Gow, N. A., Levitz, S. M., Netea, M. G., & White, T. C. (2012). Hidden killers: human fungal infections. Science Translational Medicine, 4(165), 165rv13-165rv13.
9. Nucci, M., Marr, K. A., Queiroz-Telles, F., Martins, C. A., Trabasso, P., Costa, S., & Anaissie, E. (2004). Fusarium infection in hematopoietic stem cell transplant recipients. Clinical Infectious Diseases, 38(9), 1237-1242.
10. Mookherjee, N., Anderson, M. A., Haagsman, H. P., & Davidson, D. J. (2020). Antimicrobial host defence peptides: functions and clinical potential. Nature Reviews Drug Discovery, 19(5), 311-332.

11. Denning, D. W., Riniotis, K., Dobrashian, R., & Sambatakou, H. (2003). Chronic cavitary and fibrosing pulmonary and pleural aspergillosis: case series, proposed nomenclature change, and review. Clinical Infectious Diseases, 37(Supplement_3), S265-S280.
12. Kharitonov, S. A., & Barnes, P. J. (2001). Exhaled markers of pulmonary disease. American Journal of Respiratory and Critical Care Medicine, 163(7), 1693-1722.
13. Polverino, E., Dimakou, K., Hurst, J., Martinez-Garcia, M. A., Miravitlles, M., Paggiaro & Chalmers, J. D. (2018). The overlap between bronchiectasis and chronic airway diseases: state of the art and future directions. European Respiratory Journal, 52(3).
14. Palmieri, F., Koutsokera, A., Bernasconi, E., Junier, P., von Garnier, C., & Ubags, N. (2022). Recent Advances in Fungal Infections: From Lung Ecology to Therapeutic Strategies With a Focus on Aspergillus spp. Frontiers in Medicine, 9, 832510.
15. Biswas, D., Agarwal, S., Sindhwani, G., & Rawat, J. (2010). Fungal colonization in patients with chronic respiratory diseases from Himalayan region of India. Annals of Clinical Microbiology and Antimicrobials, 9(1), 1-7.
16. Li, Z., Lu, G., & Meng, G. (2019). Pathogenic fungal infection in the lung. Frontiers in Immunology, 10, 1524.
17. Rabe, K. F., Hurd, S., Anzueto, A., Barnes, P. J., Buist, S. A., Calverley, P., ... & Zielinski, J. (2007). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. American Journal of Respiratory and Critical Care Medicine, 176(6), 532-555.
18. Ahmed, M. M., Farghaly, A. A., Raafat, R. H., & Abd Elsattar, W. M. (2019). Study of the prevalence and pattern of fungal pneumonias in respiratory intensive care units. Egyptian Journal of Bronchology, 13, 545-550.
19. Ayuk, A. C., Ekop, E., Ozoya, O., Lawal, O., & Emole, J. (2021). Prevalence and predictors of pulmonary fungal infections in patients with acute leukemia and aggressive lymphomas: Implications for cancer care in developing countries. Journal of the Pan African Thoracic Society, 2(3), 154-160.
20. Arastehfar, A., Carvalho, A., van de Veerdonk, F. L., Jenks, J. D., Koehler, P., Krause, R., ... & Hoenigl, M. (2020). COVID-19 associated pulmonary aspergillosis (CAPA)—from immunology to treatment. Journal of Fungi, 6(2), 91.
21. Huerta, A., Soler, N., Esperatti, M., Guerrero, M., Menendez, R., Gimeno, A., ... & Torres, A. (2014). Importance of Aspergillus spp. isolation in Acute exacerbations of severe COPD: prevalence, factors and follow-up: the FUNGI-COPD study. Respiratory Research, 15(1), 1-9.
22. Shahi, M., Mousavi, S. A., Nabili, M., Aliyali, M., Khodavaisy, S., & Badali, H. (2015). Aspergillus colonization in patients with chronic obstructive pulmonary disease. Current Medical Mycology, 1(3), 45.
23. Kelly, B. T., Pennington, K. M., & Limper, A. H. (2020). Advances in the diagnosis of fungal pneumonias. Expert Review of Respiratory Medicine, 14(7), 703-714.
24. Lahmer, T., Peçanha-Pietrobom, P. M., Schmid, R. M., & Colombo, A. L. (2022). Invasive fungal infections in acute and chronic liver impairment: A systematic review. Mycoses, 65(2), 140-151.
25. Phukan, A. C., Sarmabordoloi, J. N., & Mahanta, J. (2000). Bronchopulmonary candidiasis in a tertiary referral hospital of Assam, India. Indian Journal of Medical Sciences, 54(11), 491-494.
26. Skiada, A., Lass-Floerl, C., Klimko, N., Ibrahim, A., Roilides, E., & Petrikkos, G. (2018). Challenges in the diagnosis and treatment of mucormycosis. Medical Mycology, 56(suppl_1), S93-S101.
27. Cornillet, A., Camus, C., Nimubona, S., Gandemer, V., Tattevin, P., Belleguic, C., ... & Gangneux, J. P. (2006). Comparison of epidemiological, clinical, and biological features of invasive aspergillosis in neutropenic and nonneutropenic patients: a 6-year survey. Clinical Infectious Diseases, 43(5), 577-584.
28. Rafat, Z., Hashemi, S. J., Ashrafi, K., Nikokar, I., Jafari, A., Foroushani, A. R., ... & Najar-Shahri, N. (2020). Epidemiology, laboratory diagnosis and clinical aspects of fungal pulmonary infections in 384 patients hospitalized in pulmonary units in Guilan province, Iran. Iranian Journal of Microbiology, 12(4), 353.
29. Kurhade, A. M., Deshmukh, J. M., Fule, R. P., Chande, C., & Akulwar, S. (2002). Mycological and serological study of pulmonary aspergillosis in central India. Indian Journal of Medical Microbiology, 20(3), 141-144.
30. Shahid, M., Malik, A., & Bhargava, R. (2001). Prevalence of Aspergillosis in chronic lung diseases. Indian Journal of Medical Microbiology, 19(4), 201-205.
31. Jain, S., Das, S., Gupta, N., & Malik, J. N. (2013). Frequency of fungal isolation and antifungal susceptibility pattern of the fungal isolates from nasal polyps of chronic rhinosinusitis patients at a tertiary care centre in north India. Medical Mycology, 51(2), 164-169.
32. Lao, M., Li, C., Li, J., Chen, D., Ding, M., & Gong, Y. (2020). Opportunistic invasive fungal disease in patients with type 2 diabetes mellitus from Southern China: clinical features and associated factors. Journal of Diabetes Investigation, 11(3), 731-744.

33. Chakrabarti, A., Roy, P., Kumar, D., Sharma, B. K., Chugh, K. S., & Panigrahi, D. (1994). Evaluation of three serological tests for detection of anti-candidal antibodies in diagnosis of invasive candidiasis. Mycopathologia, 126, 3-7.
34. Rayens, E., & Norris, K. A. (2022, January). Prevalence and healthcare burden of fungal infections in the United States, 2018. In Open Forum Infectious Diseases (Vol. 9, No. 1, p. ofab593). US: Oxford University Press.
35. Berkow, E. L., & Lockhart, S. R. (2017). Fluconazole resistance in Candida species: a current perspective. Infection and Drug Resistance, 237-245.
36. Kolwijck, E., & van de Veerdonk, F. L. (2014). The potential impact of the pulmonary microbiome on immunopathogenesis of Aspergillus-related lung disease. European Journal of Immunology, 44(11), 3156-3165
37. Berkow, E. L., Lockhart, S. R., & Ostrosky-Zeichner, L. (2019). Antifungal Susceptibility Testing: Current Approaches. Clinical Microbiology Reviews, 32(4), e00069-19.
38. Lim, J. J., Grinstein, S., & Roth, Z. (2017). Diversity and Versatility of Phagocytosis: Roles in Innate Immunity, Tissue Remodeling, and Homeostasis.
39. Chung, E. Y., Kim, S. J., & Ma, X. J. (2006). Regulation of cytokine production during phagocytosis of apoptotic cells. Cell Research, 16(6), 537-543.
40. Lacy, P., & Stow, J. L. (2011). Cytokine release from innate immune cells: association with diverse membrane trafficking pathways. Blood, 118(1), 9–18.