PREVALENCE OF FILAMENTOUS FUNGI IN RESPIRATORY TRACT INFECTED PATIENTS WITH NEGATIVE ACID-FAST BACILLI SMEAR TEST IN LAGOS, NIGERIA

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Abstract

Background: The idea of commensal relationship between fungi and infections of the lungs which is a major organ of the respiratory system has been suggested in the past. Patients with filamentous fungi in their airways often present with sign and symptoms mimicking tuberculosis.

Aim: This study aims at determining the prevalence of filamentous fungi in acid-fast bacilli smear negative sputum from patients with respiratory tract infection in Lagos, Nigeria.

Materials and Methods: A total of 360 sputum samples of patients with respiratory tract infection were collected. These samples were subjected to mycological investigations using microscopy and culture. The sputum samples were cultured onto Sabouraud Dextrose Agar (SDA) to obtain fungal isolates. The fungal isolates were tested for their susceptibility using disk diffusion method and the zones of inhibition were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) guidelines.

Results: The results revealed a prevalence of 75.48% for *Aspergillus* species in the samples collected. *Aspergillus flavus* (36.7%), *Aspergillus niger* (18.7%), *Aspergillus fumigatus* (1.4%), *Aspergillus wentii* (17.64%) and *Aspergillus aculeatus* (1.04%) were the predominant species of *Aspergillus* spp. isolated from the sputum samples collected. Other filamentous fungi isolated include *Rhizopus* spp. And *Penicillium* spp. The study showed the higher prevalence of *Aspergillus* isolates among females (58.82%) compared to males (41.18%). The age group 30-39 had the highest percentage of occurrence of filamentous fungi (25.3%) among the different age groups encountered in the study.

Conclusion: The identified fungal species in this study is a proof of mold-causing infection. It can be concluded that the present study helps in establishing that *Aspergillus* species are responsible for the respiratory tract infection in the sputum analyzed

Keywords: Filamentous fungi, antifungal assay, respiratory tract infection, sputum, gender, age group

INTRODUCTION

Respiratory tract infections are commonly responsible for one-third of infectious disease associated mortality, accounting for about 4.3 million annual deaths amongst which fungal infections of the respiratory tract are largely unrecognized, and the true problem is hard to pin down (Chowdhary *et al.*, 2016). Despite treatment, most invasive fungal infections are associated with high mortality rates of >50% (Agarwal *et al.*, 2010; Chowdhary *et al.*, 2016).

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A number of uncommon filamentous fungi, such as *Scedosporium*, *Fusarium*, *Penicillium*, melanized molds, and basidiomycetes, have emerged as etiological agents of well-characterized respiratory disorders over the years (Denning *et al*., 2006; Ogawa *et al*., 2009; Singh *et al*., 2013; Chowdhary *et al*., 2014).

Filamentous fungi are ubiquitous, contaminating soil, air, food and other substrates and due to their wide distribution, they have medical and economic implications. Irrespective of their use as a source of antibiotics, vitamins and raw materials for various industrially important chemicals, most filamentous fungi produce metabolites associated with a variety of health risks in humans (Egbuta *et al*., 2017). They are responsible for diseases in humans and require specific host conditions to be met before infection can occur (Powers-Fletcher *et al.,* 2016).

Also, emergence of resistance in filamentous fungi to azole antifungal drugs used as basis of therapy is another challenging development observed in the last two decades. This emerging problem is mainly attributed to the widespread usage of azole fungicides in the environment for agricultural and material preservation practices (Chowdhary *et al*., 2013; Chowdhary *et al.,* 2016).

Aspergillosis is a disease caused by an exceedingly common mold, of the genus *Aspergillus*. They are known to cause disease in immuno-compromised individuals. It is a disease which clinically resembles tuberculosis that it usually misdiagnosed as tuberculosis. Recent studies have shown that *Aspergillus* infection can result in a broad range of airway complications with features mimicking those of Tuberculosis (Mwaura *et al*., 2013).

Acid-Fast Bacilli (AFB) tests are most often used to diagnose an active tuberculosis (TB) infection. The tests look for the presence of AFB bacteria in the sputum (Egbe *et al*., 2010). This test can be done by the smear method or the culture method of sputum samples. Sputum is pus that accumulates deep within the lungs of a patient with pneumonia, tuberculosis (TB), or other lower respiratory infection.

The aim of this study is to negate the impression that co-existing fungi are harmless, saprophytic invaders, and to advance added data to support whether an infection of the respiratory system is caused by pathogenic filamentous fungi or not and to estimate the prevalence of filamentous fungi in acid-fast bacilli smear negative sputum from patients with respiratory tract infection in Lagos, Nigeria.

RESEARCH HYPOTHESIS

Patients with filamentous fungi in their airways often present with sign and symptoms mimicking tuberculosis

MATERIALS AND METHODS

Study Design and Population

Sputum of patients that has tested negative to acid-fast bacilli (AFB) smear at hospitals were collected from two tertiary health institutions in Lagos State, Lagos University Teaching Hospital (LUTH), Idi- araba and Lagos Mainland Hospital, Yaba and screened for filamentous fungi. These samples have been pre-treated by medical laboratory scientists at each hospital to rule - out tuberculosis. The patients were selected on the basis of a category of smear negativity for AFB.

Sample Size Calculation

The sample size for the study was calculated using the prevalence (37.69%) reported by Gutch *et al*. (2015). The sample size was calculated as:

$$\mathbf{N = Z^2 P \, X \, \frac{1 - P}{D^2}}$$

Where,

N= Sample size

Z= 1.96

P=37.69%

D= 0.05

Therefore, N=360.87

Thus, 360 (180 samples from each hospital) sputa were analysed for this study.

Ethical Consideration

Ethical approval was obtained from the Health Research and Ethics Committee of the Lagos State University Teaching Hospital (Reference No. CMUL/HREC/03/22/1035). All patient's data were anonymized and handled only by authorized personnel in order to ensure confidentiality.

Sample Collection

Samples were collected weekly for eighteen consecutive weeks (July, 2022 to October, 2022) from two tertiary hospitals in Lagos. These were collected in sterile bottles by medical personnel in each hospital and labelled. The samples were stored on ice, and processed within 2 hours of collection. A total of 360 sputum samples from 360 patients suffering from respiratory infection were analyzed.

Isolation and identification of fungal isolates

The sputum samples were cultured on Sabouraud Dextrose Agar (SDA, Oxoid, Basingstoke, England) supplemented with 50mg/ml chloramphenicol to permit the successful isolation of fungi. The cultures were incubated at 26ºC (Pashley *et al*., 2012) for 48 hours . Plates were observed daily for any macroscopic characteristics. Then, a little portion of the growing colony was teased with a sterile inoculation needle and put on a clean microscope slide in a drop of Methyl-Blue. This was placed under a cover slip and compressed with the inoculation needle's butt before the extra fluid was blotted off. The preparation was examined under a light microscope with an attached camera (Motic McCamera [2000] 2.0 megapixel digital coloured camera) connected to a computer, for the microscopic photograph of the fungi. Both macroscopic and microscopic characteristics were compared with those in a standard mycology text books (Kidd *et al*., 2016; Kibbler *et al.,* 2017).

Antifungal Susceptibility Testing

Disk diffusion test method was employed to yield a quantitative result (zones of inhibition in millimeters) and a qualitative interpretive category (e.g., susceptible or resistant) and to track the *in-vitro* antifungal susceptibility in filamentous fungi according to the Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2020). The antifungal agents used in this study are: Fluconazole (FCA), Itraconazole (ITC), Nystatin (NY) and Ketoconazole (KTA). All these were purchased commercially.

Statistical Analysis

The data obtained from the study were represented in graphs using Microsoft Excel Spreadsheet.

RESULTS

Out of the 360 samples, 154 (42.78%) were collected from male while the remaining 206 (57.22%) were from females both within the age range of 10-89 years as shown in Table 1.

Based on the morphological comparison and photomicrograph characteristics with the confirmed representatives of standard mycology text books, (Kidd *et al*. 2016 and Kibbler, *et al.* 2017). Forty-one samples did not yield any fungal growth, 289 yielded 7 morphologically different species of filamentous fungi while the remaining 30 samples yielded yeasts. The fungal isolates were identified as *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus wentii, Aspergillus aculeatus, Rhizopus sp.* and *Penicillium* sp. as shown in Figure 1 and on Table 2

Of the 289 isolates, 170 (58.82%) were from females while the remaining 119 (41.18%) were from males (Figure 2). The occurrence of all isolated filamentous fungi varied across age and gender as shown in Figures 3 and 4 respectively. The predominant species isolated were *Aspergillus flavus* (36.7%), *Aspergillus niger* (18.7%), *Aspergillus fumigatus* (1.4%), *Aspergillus wentii* (17.64%), *Aspergillus aculeatus* (1.04%), *Rhizopus* spp. (5.5%) and *Penicillium* sp. (19.02%) as shown on Table 2.

The susceptibility assay was carried out on the seven identified filamentous fungi using disk diffusion. (Table 3). The zones of inhibition were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) guidelines.

Table 1: Age and gender distribution of the study population

	Gender	
Age (Years)	**Female Frequency (%)**	**Male Frequency (%)**
10-19	12 (3.3%)	8 (2.3%)
20-29	50 (13.9%)	41 (11.4%)
30-39	52 (14.4%)	35 (9.7%)
40-49	40 (11.1%)	37 (10.3%)
50-59	17 (4.7%)	17 (4.7%)
60-69	19 (5.2%)	6 (1.7%)
70-79	10 (2.7%)	6 (1.7%)
80-89	3 (0.8%)	7 (1.9%)

Table 2: Filamentous fungal isolated from sputum of patients suffering from respiratory tract infection

Fungal isolates	Frequency (%)
Aspergillus aculeatus	3 (1.04%)
Aspergillus flavus	106 (36.7%)
Aspergillus fumigatus	4 (1.4%)
Aspergillus niger	54 (18.7%)
Aspergillus wentii	51 (17.64%)
Penicillium sp.	55 (19.02%)
Rhizopus sp.	16 (5.5%)

Table 3: Antifungal susceptibility breakpoints of filamentous fungi isolates against antifungal disk

	Antifungal agents/ Diameter of inhibition zone (mm)			
	Fluconazole (25µg)	**Itraconazole (50µg)**	**Ketoconazole (15µg)**	**Nystatin (100µg)**
Aspergillus flavus	00 (R)	20 (S)	21 (S)	20 (S)
Aspergillus niger	00 (R)	25 (S)	10 (R)	25 (S)
A. fumigatus	25 (S)	25 (S)	23 (S)	25 (S)
A. wentii	00 (R)	21 (S)	21 (S)	20 (S)
A. aculeatus	00 (R)	20 (S)	20 (S)	14 (R)
Rhizopus sp.	00 (R)	24 (S)	16 (I)	09 (R)
Penicillium sp.	00 (R)	24 (S)	22 (S)	17 (I)

S – Susceptible (≥19mm), R – Resistant (≤ 14mm), I – Intermediate (15 to 18mm)

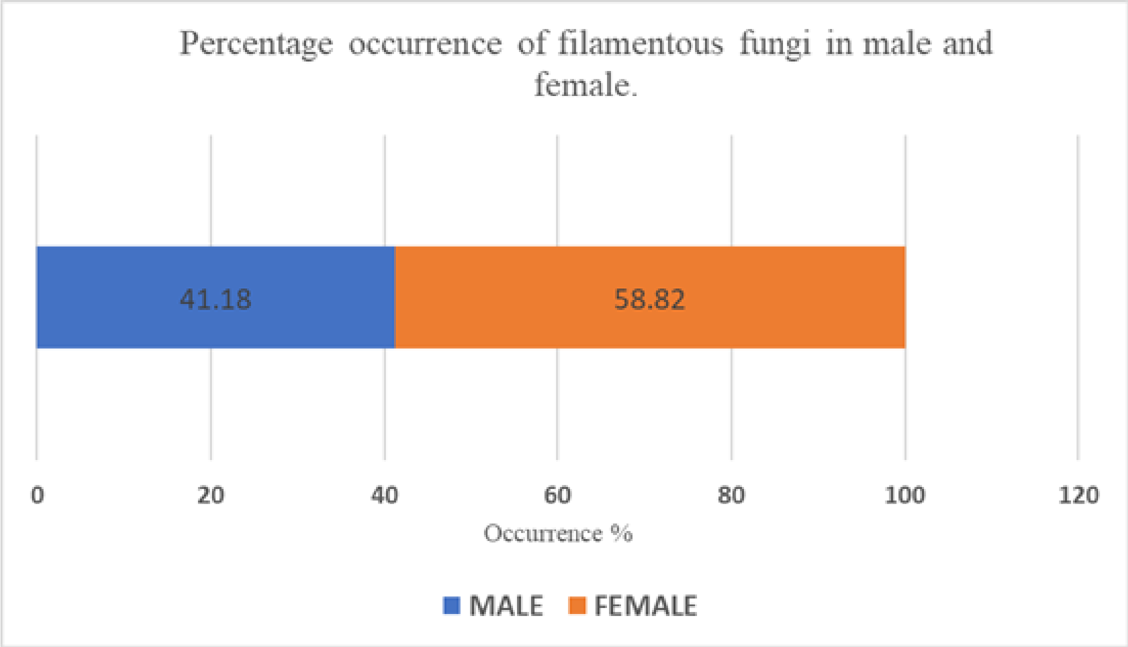

Figure 2. Percentage occurrence of filamentous fungi in male and female

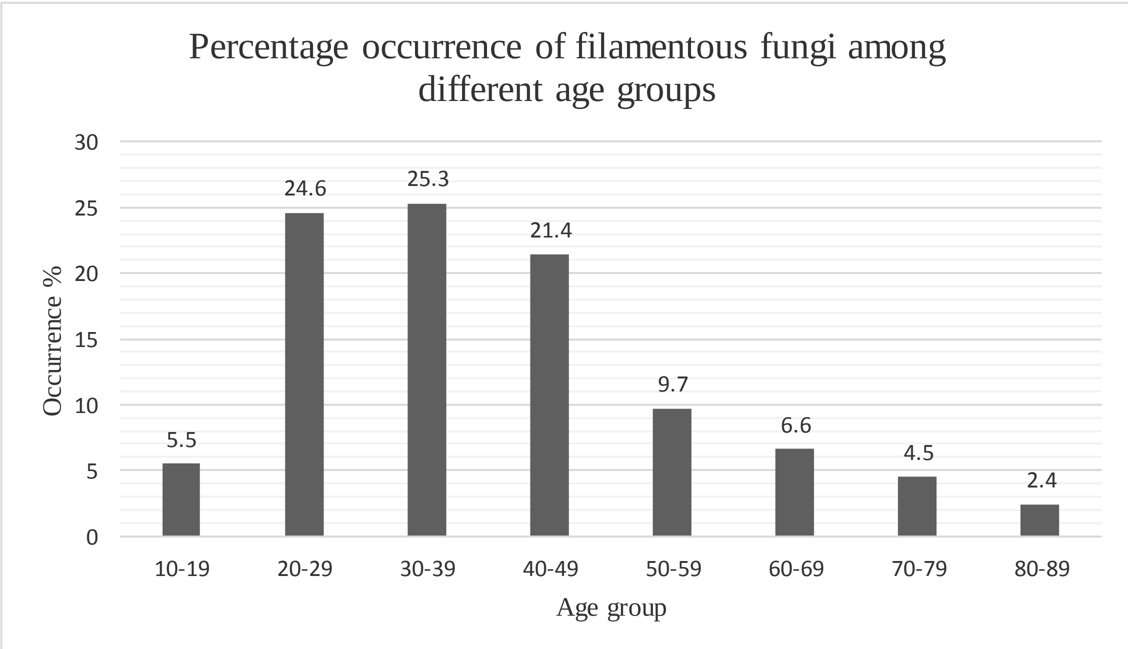

Figure 3. Percentage occurrence of filamentous fungi among different age groups

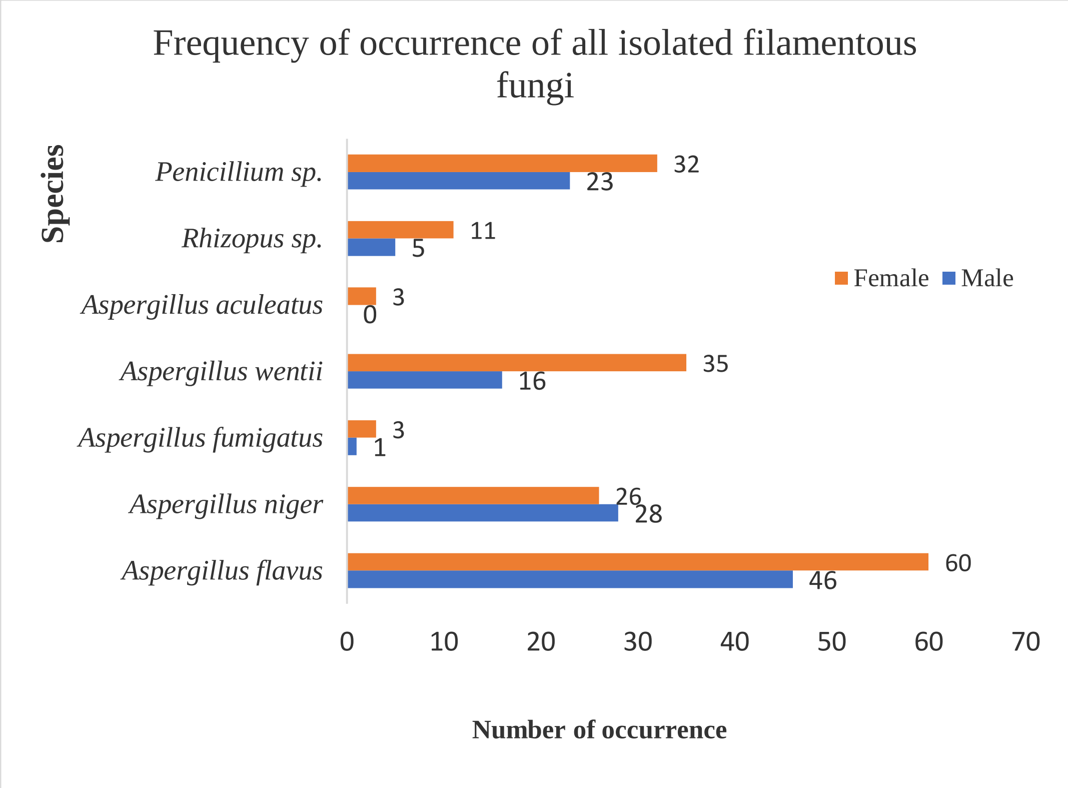

Figure 4. Frequency of occurrence of all isolated filamentous fungi across genders

DISCUSSION

The survey result in this present study revealed that filamentous fungi can occur irrespective of age and gender of people and it can be the causative agent of most respiratory tract infections.

The frequency of filamentous fungi encountered in this study is higher in female patients than in the male patients at 58.82 % and 41.18 % respectively. However, Bansod and Rai (2008) reported 46 % (203 out of 500 samples) mycotic infections in pulmonary infection patients. They observed that 68.8 % males and 46.6 % females were culture positive. This could be due to the difference in research locations.

In this study, age group 30 to 39 has the highest occurrence of filamentous fungi of 25.3% followed by the age group 20 to 29 with the occurrence of 24.6%. This findings are similar to the report of Ekenna *et al.* (2007) that *Aspergillus* sp. (34.3%), *Penicillium* sp. (7%), *Rhizopus* sp. (2.9%) had the highest occurrence within the age group 20-39.

This study showed that there is higher percentage of occurrence in *Aspergillus flavus* (36.7%) which is in accordance with the research of Saunders *et al*. (2011) who reported that the isolation rate of *Aspergillus sp*. from respiratory specimens varies widely from 6 to 58%. Dabo and Yusha'u (2007) also reported that 10.59 % patients tested positive for systemic fungal infection caused by filamentous fungi in patients with suspected tuberculosis in Nigeria. This implies that respiratory tract infection is associated with species identified.

In this study, only *Aspergillus fumigatus* was susceptible to Fluconazole (25 µg) while all other organism showed no zone of inhibition which means they are resistant to Fluconazole (25 µg). This is in conformity with the findings of Alastruey-Izquierdo *et al.* (2009) who reported that the use of azoles in agricultural products consumed by humans has been described as a cause of the emergence of triazole resistant in *Aspergillus fumigatus* isolates and other filamentous fungi are intrinsically resistant to some antifungals. They also stated that the members of the order Mucorales comprises several pathogenic species that are resistant to voriconazole.

CONCLUSION

The identified fungal species in this study is a proof of mold-causing infection. With the high prevalence of *Aspergillus* species (75.48%) in the samples collected, it can be concluded that the present study helps in establishing that *Aspergillus* species are responsible for the respiratory tract infection in the sputum analyzed which can result in a broad range of airway complications with features mimicking those of Tuberculosis.

REFERENCES

Agarwal, R., Hazarika, B., Gupta, D., Agarwal, A.N. and Chakrabarti, A. (2010). *Aspergillus* hypersensitivity in patients with chronic obstructive pulmonary disease: COPD as a risk factor for ABPA. *Medical Mycol*ogy, **48**: 988-994.

Alastruey-izquierdo, A., Marcia S.C., Melhem, L., Bonfiett, I.X. and Rodriguez-Tudela, J.L. (2015). Susceptibility test for fungi: clinical and laboratorial correlations in medical mycology. *Clinical Laboratory Standards Institute (CLSI)*, **57**(19): 57-64.

Alexopolous, C.J., Mims, C.W. and Blackwell, M. (2007). Introductory Mycology. Fourth edition. Wiley India, New Delhi. Pp 869.

Bansod, S. and Rai M. (2008). Emerging of Mycotic infection in patients infected with Mycobacterium tuberculosis. *World Journal of Medical Sciences*, **3**(2): 74- 80.

Chowdhary, A., Agarwal, K., and Meis, J. F. (2016). Filamentous Fungi in Respiratory Infections. What Lies Beyond Aspergillosis and Mucormycosis? *PLOS Pathogens*, **12**(4): 1-7.

Chowdhary, A., Kathuria, S., Agarwal, K. and Meis, J.F. (2014). Recognizing filamentous basidiomycetes as agents of human disease: A review. *Medical Mycology*, **52**: 782-797.

Chowdhary, A., Kathuria, S., Xu, J. and Meis, J.F. (2013). Emergence of azole-resistant *Aspergillus fumigatus* strains due to agricultural azole use creates an increasing threat to human health. *PLOS Pathogen*, **2**: 32-38.

Dabo, N.T. and Yusha'u, M. (2007). Incidence of systemic mycoses in patients with suspected tuberculosis in Kano, Nigeria. *International Journal of Pure and Applied Sciences*, **1**(1): 35-39.

Denning, D.W., O'Driscoll, B.R., Hogaboam, C.M., Bowyer, P. and Niven, R.M. (2006). The link between fungi and severe asthma: a summary of the evidence. *European Respiratory Journal,* **27**: 615-626.

Denning, D.W., Pleuvry, A and Cole, D.C. (2011). Global burden of chronic pulmonary aspergillosis as a sequel to tuberculosis. *Bulletin of World Health Organization*, **89**: 864-872.

Egbe, C. A., Ndiokwere, C. and Omoregie, R. (2010). Microbiology of Lower Respiratory Tract Infections in Benin City, Nigeria. *Malaysian Journal of Medicine*, **18**(2): 27-31.

Egbuta, M.A., Mwanza, M. and Babalola, O.O. (2017). Health Risks Associated with Exposure to Filamentous Fungi. *International Journal of Environmental Research and Public Health*, **14**: 1-17.

Ekenna, O., Uba, A., Chikwem, J.O., Mambula, S., Aliyu, M.B. and Mohammed I. (2007). Relevance of moldy fungi as agents of chronic lower respiratory tract infection in patients seen in Maiduguri, Nigeria. *West African Journal of Medicine*, **26**(1): 117-120.

Ellis, D., Davis, S., Alexiou, H., Handke, R. and Bartley, R. (2007). Descriptions of medical fungi, 2nd Edition. Nexus Print Solutions, Underdale, South Australia. Pp 198.

Espinel-Ingroff, A., Chakrabarti, A., Chowdhary, A., Cordoba, S., Dannaoui E, Dufresne, P., Fothergill, A., Ghannoum, M., Gonzalez, G.M., Guarro, J., Kidd, S., Lass-Flörl, C., Meis, J.F., Pelaez, T, Tortorano, A.M. and Turnidge, J. (2015). Multicenter evaluation of MIC distributions for epidemiologic cutoff value definition to detect amphotericin B, posaconazole, and itraconazole resistance among the most clinically relevant species of Mucorales. *Antimicrobial Agents Chemotherapy*, **59**: 1745-1750.

Gutch, R. S., Yadu, R., Nawange, S. R., Singh, S.M., Gumasta, R., Nawange, M. and Kavishwar, A. (2015). Prevalence of opportunistic fungal infection in patients with pulmonary tuberculosis in Madhya Pradesh, Central India. *Microbioz Journals, Journal of Microbiology and Biomedical Research,* **1**(6): 2395-5678.

Kidd, S., Halliday, C., Alexiou, H and Ellis, H. (2016). *Descriptions of medical fungi.* Third edition. Newstyle Printing. Pp 296.

Kibbler, C.C. Barton, R., Gow, N.A.R. ,Howell, S., MacCallum, D.M. and Manuel, R. J. (2017). *Oxford Textbook of Medical Mycology*. Oxford University Press. Pp 266-274.

Mandell, L.A., Wunderink, R.G., Anzueto, A., Bartlett, J.G., Campbell, G.D., Dean, N.C., Dowell, S.F., File, T.M., Musher, D.M., Niederman, M.S., Torres, A. and Whitney, C.G. (2007). Infectious Diseases Society of America, American Thoracic Society. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clinical Infectious Disease*, **44**(2): 27-72.

Mwaura, E.N., Matiru, V. and Bii, C. (2013). Mycological Findings of Sputum Samples from Pulmonary Tuberculosis Patients Attending TB Clinic in Nairobi, Kenya. *Virology and Mycology*, **2**(3): 1-6.

Ogawa, H., Fujimura, M., Takeuchi, Y. and Makimura, K. (2009). Efficacy of itraconazole in the treatment of patients with chronic cough whose sputa yield basidiomycetous fungi-Fungus-associated chronic cough (FACC). *Journal of Asthma*, **46**: 407-412.

Ogawa, H., Fujimura, M., Takeuchi, Y., Makimura, K. and Satoh, K. (2011). Sensitization to *Bjerkandera adusta* enhances severity of cough symptom in patients with fungus-associated chronic cough (FACC). *Journal of Medical Mycology*, **52**: 205-212.

Pashley, C H. , Fairs, A., Morley, J. P., Tailor, S., Agbetile, J., Bafadhel, M., Brightling, C.E. and Wardlaw, A.J. (2012). Routine processing procedures for isolating filamentous fungi from respiratory sputum samples may underestimate fungal prevalence. *Medical Mycology*, **50**: 433-438.

Pavord, I.D., Pizzichini, M.M., Pizzichini, E. and Hargreave, F.E. (1997). The use of induced sputum to investigate airway inflammation. *Thorax*, **52**: 498-501.

Performance Standard for Antimicrobial Susceptibility Testing (2020). 30th Edition, CLSI supplement M100Wayne, PA: Clinical and Laboratory Standards Institute, Pp 332.

Powers-Fletcher, M. V., Kendall, B. A., Griffin A. T. and Hanson, K. E. (2016). Filamentous fungi. *Microbiology Spectrum*, **4**(3): 1-29.

Saunders R, Modha D, Claydon A. and Gaillard E. (2011). Chronic *A. fumigatus* colonization of the cystic fibrosis airway is common and may be associated with a more rapid decline in lung function. *Journal of Cystic Fibrosis*, **10**: 537-543.

Singh, P.K., Kathuria, S, Agarwal, K., Gaur, S.N. and Meis, J.F. (2013). Clinical significance and molecular characterization of nonsporulating molds isolated from the respiratory tracts of bronchopulmonary mycosis patients with special reference to basidiomycetes. *Journal of Clinical Microbiology*, **51**: 3331-3337.