DETECTION AND PREVALENCE OF *ASPERGILLUS* SPECIES RESPONSIBLE FOR RESPIRATORY TRACT INFECTION AMONG PATIENTS WITH NEGATIVE ACID-FAST BACILLI SMEAR IN LAGOS, NIGERIA

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Abstract

Aspergillus is a common filamentous fungus which is ubiquitous and are known to be responsible for diseases in humans. Respiratory infections are commonly responsible for most infectious disease associated mortality, amongst which the respiratory tract fungal infections are mostly unknown, and the real problem is hard to pin down. Patients with filamentous fungi in their airways often present with sign and symptoms mimicking tuberculosis. Acid-Fast Bacilli (AFB) tests are often utilized in diagnosing an active tuberculosis (TB) infection. This present study was conducted to detect and estimate the prevalence of Aspergillus species responsible for respiratory tract infection among patients with negative Acid-Fast Bacilli smear in Lagos, Nigeria. A total of 360 sputum samples of patients with respiratory infection were collected and subjected to mycological examination using culture and microscopy. The results revealed a prevalence of 60.5% 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 (0.8%) were the predominant species of Aspergillus isolated. The study showed a higher prevalence of Aspergillus isolates among females (35.2%) compared to males (25.3%) and the age group 20-29 emerged with the highest percentage of occurrence (15.6%) amongst the various age groups in the study. This study helps in establishing that Aspergillus species are responsible for the respiratory infection in the sputum analyzed and this result is expected to assist in the management of patients presenting respiratory infections.

Keywords: *Aspergillus, filamentous fungi, respiratory infection, sputum, gender, age group*

Introduction

Respiratory infections are commonly responsible for most death-associated infectious disease, accounting for about 4 million or more deaths annually amongst which the respiratory tract fungal infections are mostly unknown, and the real problem is hard to pin down (Chowdhary *et al.*, 2016). High mortality rates are associated with most invasive fungal infections estimated to be about 50% or greater regardless of treatment (Agarwal *et al.*, 2010; Chowdhary *et al.*, 2016). Some exceptional filamentous fungi, for instance *Penicillium*, *Scedosporium*, *Fusarium*, molds, and basidiomycetes, have developed as etiological agents of well- typified respiratory illnesses over the years. (Denning *et al*., 2006; Ogawa *et al*., 2009; Singh *et al.*, 2013; Chowdhary *et al*., 2014).

Filamentous fungi are ubiquitous, they contaminate food, air soil and other substrates and they have medical and economic implications due to their extensive dispersal. Most filamentous fungi produce metabolites associated with a variety of health risks in humans irrespective of their use as a source of vitamins, antibiotics and raw materials for various industrially important chemicals (Egbuta *et al.*, 2017). Before infection can occur, certain host preconditions must be met as these filamentous fungi are known to be responsible for diseases in humans (Powers-Fletcher *et al.*, 2016). Patients with filamentous fungi such as *Aspergillus* in their airways often present with sign and symptoms mimicking tuberculosis and Acid-Fast Bacilli (AFB) tests are often utilized in diagnosing an active tuberculosis (TB) infection. These tests scan for the presence of AFB bacteria in the sputum and can be done by the smear method or the culture method of sputum samples. Sputum is pus that amasses deep within the lungs of a patient with pneumonia, tuberculosis (TB), or other lower respiratory infection (Egbe *et al*., 2010).

Aspergillus species pose noteworthy risk to the host whose immune system has been compromised. Infections with *Aspergillus* species cause a wide array of disease which is collectively referred to as aspergillosis. The most frequent sites of infection are the lung and respiratory tract, but can spread to other parts of the human system (Diba *et al.*, 2007). No less than 30 species of the genus *Aspergillus* have been associated with human diseases, and Invasive

Aspergillosis (IA) has been reported to be frequently caused by *Aspergillus fumigatus* but in some study, *Aspergillus tereus* is more common. Mostly, identification of the *Aspergillus* species is centered on the morphological or phenotypic features of the colony and microscopic examinations (McClenny, 2005). This study is aimed at detecting whether infection of the respiratory system is caused by a pathogenic filamentous fungus, *Aspergillus* or not and also to estimate prevalence of filamentous fungi in Acid-Fast Bacilli smear negative sputum from patients with respiratory infection in Lagos, Nigeria.

Materials and methods

Study Design and Population

Sputum of patients that have tested negative to acid-fast bacilli (AFB) smear at hospitals were collected from 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 of the named hospitals to rule - out tuberculosis. The patients were chosen 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:

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

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.

Exclusion Criteria

Patients with positive samples of Acid-Fast Bacilli were excluded from the study

Inclusion Criteria

Patients of all ages with Acid-Fast Bacilli negative sputum samples.

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). To guarantee confidentiality, all patient data were anonymized and handled only by authorized personnel.

2.2 Sample Collection

Samples were collected weekly for eighteen consecutive weeks (July, 2022 - October, 2022) in sterile bottles by medical personnel in each hospital and labeled with essential data about the patient. Data such as gender and age were collected and recorded. The samples were stored on ice and processed within 2 hours of collection.

Culture Method

The sputum samples were cultured on Sabouraud dextrose agar (SDA) supplemented with 50 mg/ml chloramphenicol to permit the successful isolation of fungi (Pashley *et al*., 2012). Culture plates were examined without opening three times (after 48 hours, between days 4 and 6, and on day 7) because some morphological features can be seen quickly, while some can take up to four weeks before presenting any visible morphological attributes. Cultured plates were inspected, and the number of observable colonies was recorded at each point in time.

A total of 360 sputum samples from 360 patients suffering from respiratory infections were analyzed. Samples gotten from Lagos University Teaching Hospital were labeled LTH 1-180 while those gotten from Mainland Hospital, Yaba were labeled MHY 1-180.

Identification of isolates

Each fungal colony morphology type (by significant characteristics such as texture, topography and pigmentation) was recorded in order to accurately identify the isolated filamentous fungi. To observe the conidiophore's detailed arrangement and how their spores were produced, an inoculation needle was used to pick up a minute ration of the fungal colony and mounted in a drop of Lactophenol cotton blue on a sterile microscope slide. The minute ration of the fungal colony picked was mashed with the butt of the inoculation needle then secured with a cover slip and the excess fluid was blotted off. Photomicrograph of the morphological characteristics of the pure culture colonies were observed under a light microscope, using ocular and objective lens at 16 x 40 for their magnification. Isolated fungi were identified to a species level based on phenotypic features as described by Ellis *et al*. (2007) and Alexopolous *et al*. (2007).

Data Analysis

The distribution f study population and results obtained were presented in figures and tables using Microsoft Word and Excel 2019 edition.

Results

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

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%)
Total	**206 (57.22%)**	**154 (42.78%)**

The study revealed that *Aspergillus flavus* is the most common isolated *Aspergillus* species. Other *Aspergillus* species that were isolated includes *Aspergillus aculeatus*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus wentii* in different frequency and percentage of occurrence as shown in Table 2.

Table 2: Isolated *Aspergillus* species from sputum of patients with respiratory infection

***Aspergillus* species**	**Females Frequency (%)**	**Males Frequency (%)**	**Total Frequency (%)**
Aspergillus aculeatus	3 (0.8%)	-	**3 (0.8%)**
Aspergillus flavus	60 (16.6%)	46 (12.8%)	**106 (29.4%)**
Aspergillus fumigatus	3 (0.8%)	1 (0.3%)	**4 (1.1%)**
Aspergillus niger	26 (7.2%)	28 (7.8%)	**54 (15%)**
Aspergillus wentii	35(9.7%)	16 (4.4%)	**51 (14.1%)**
Total Frequency (%)	**127 (35.2)**	**91 (25.3%)**	**218 (60.5%)**

It was also discovered in this study that there is a higher percentage prevalence of Aspergillus species in female (35.2%) than in male (25.3%) between the age group 20-29 as shown in Figure 1 and Figure 2 respectively.

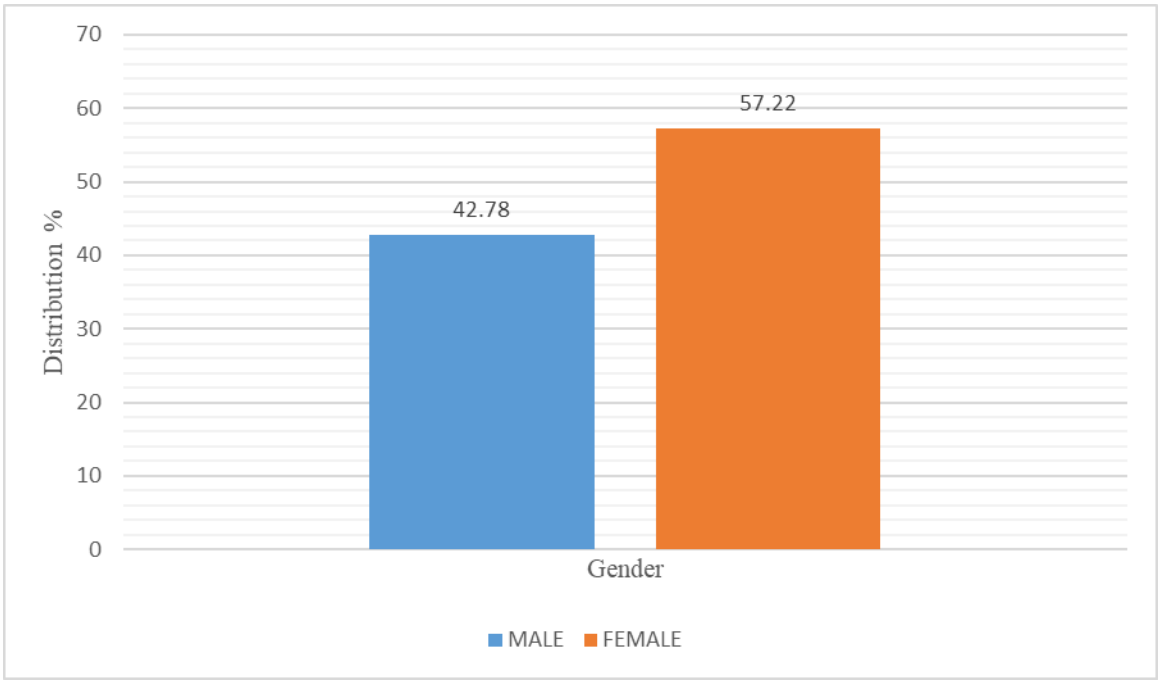

Figure 1. Percentage occurrence of *Aspergillus* species in male and female

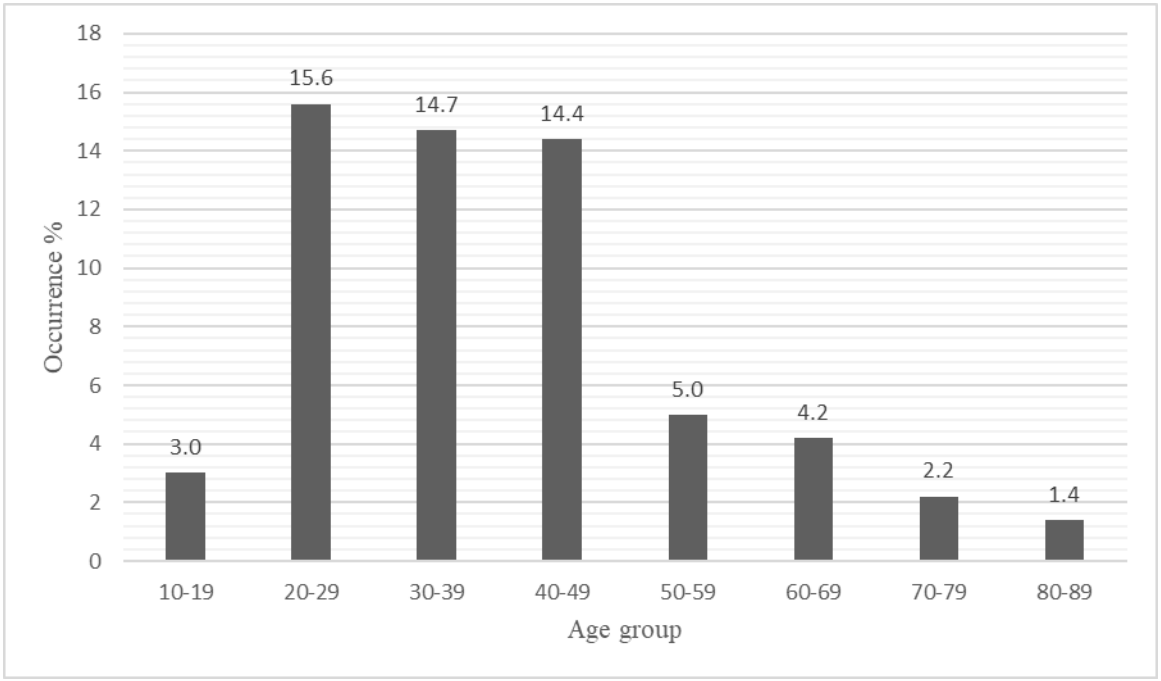

Figure 2. Percentage occurrence of *Aspergillus* species among different age groups

Figure 3 shows that *Aspergillus flavus* has the highest occurrence both in males (46 of 360) and in females (60 of 360) among the five identified *Aspergillus* species encountered in this study

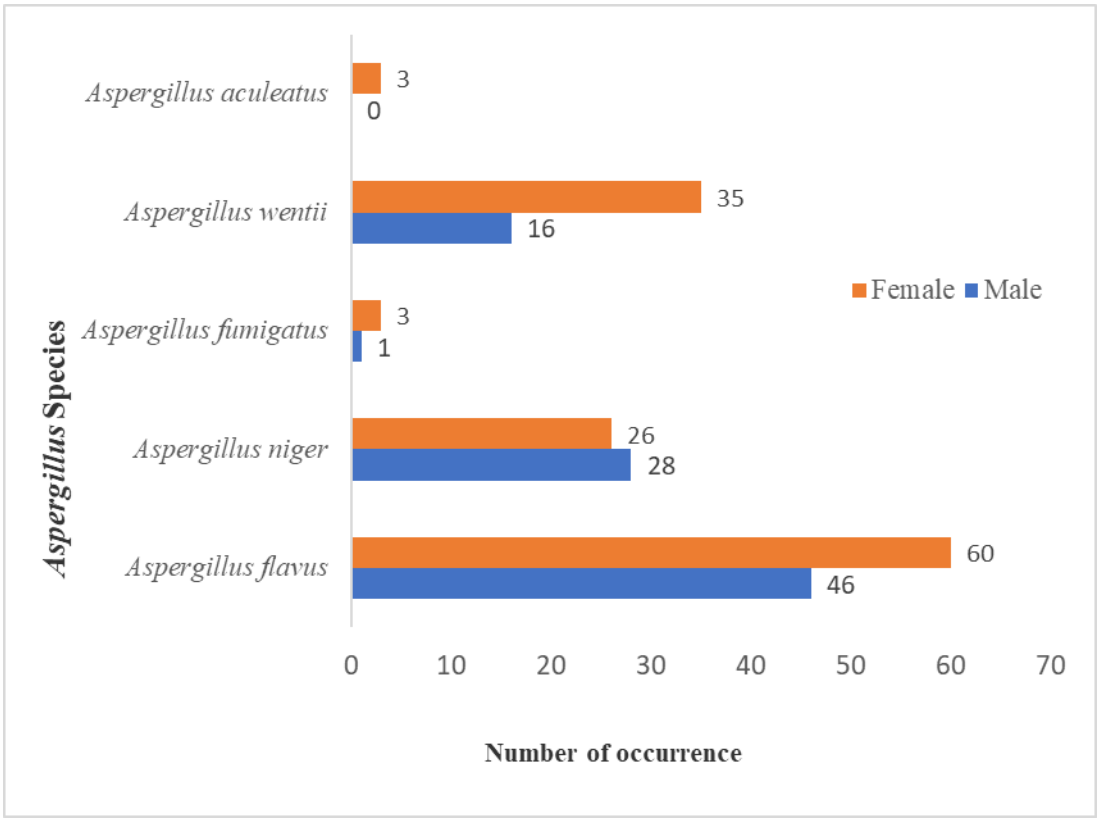

Figure 3. Frequency of occurrence of all identified *Aspergillus* species across genders

Discussion

The data obtained in this study shows that mold infection can ensue regardless of age and gender of people, and it can be the suspected causative agent of most respiratory infections as previously suggested in the report of Chowdhary *et al.* (2016). This study showed a higher prevalence of *Aspergillus* species (60.5%) isolated from the sputum samples collected from the hospitals compared to the previous reports of Diba *et al.* (2007) and Mwaura *et al*. (2013) at 25% and 2.9% respectively. This could be due to difference in population, and it can also be attributed to organism load as suggested in the report of Mwaura *et al*. (2013).

In the report of Bansod and Rai (2008), 203 out of 500 samples was positive to mycotic infections resulting in 46 % prevalence observed in patients with pulmonary infection. A higher percentage of males (68.8 %) and lower percentage of females (46.6 %) were observed to be culture positive. On the other hand, in this study, a higher percentage of *Aspergillus* species occurrence is higher in female patients than as observed in male patients encountered at 35.2 % and 25.3 % respectively. This could be due to the difference in research locations, population or that females are more likely to have respiratory infection than male as reported by Egbe *et al*. (2010) and Mwaura *et al*. (2013).

In this study, the age group 20-29 has the highest occurrence of *Aspergillus* species of 15.6%. This finding correlates with the report of Ekenna *et al.* (2007) who reported *Aspergillus* sp. occurs at 34.3% occurrence within the age group 20-39. In accordance with the research of Saunders *et al*. (2011) who reported that the prevalence of *Aspergillus* sp. isolation from respiratory samples varies usually from 6 to 58%. Result of this study showed a higher percentage of *Aspergillus flavus* (29.4%)

Dabo and Yusha'u (2007) also reported that about 10.59 % individuals tested positive for fungal infection caused by filamentous fungi in patients with suspected tuberculosis in Nigeria. This implies that respiratory infection is associated with the species identified.

The result of this study showed that *Aspergillus flavus* was the most frequent within all *Aspergillus* species identified followed by *A. niger* and *A. wentii* at 29.4%, 15% and 14.1% respectively. The rare isolated Aspergillus species were *A. fumigatus* (1.1%) and *A. aculeatus* (0.8%). This is in agreement with a mycological research data in Saudi Arabia on the *Aspergillus* flora which included *A.flavus, A.niger* and *A.fumigatus* respectively (Diba *et al.*, 2007).This finding is in contrast with the observation of Shesh Rao *et al*. (2015) that *A. fumigatus* was the predominant species isolated from respiratory specimens and *A. niger* was predominantly isolated from nasal polyps whereas Geiser (2009) and Chowdhary *et al.* (2016) reported *Aspergillus fumigatus* to be the most frequent species to be isolated from respiratory tract secretions and it was found to be responsible for most Aspergillosis cases. Other species include; *Aspergillus flavus, Aspergillus nidulans, Aspergillus terreus* and *Aspergillus niger*.

The species of the genus *Aspergillus* are known to trigger disease in individuals with suppressed or compromised immune system called Aspergillosis. It is a disease that is clinically and pathologically similar to tuberculosis which is usually not recognized and is sometimes misdiagnosed as tuberculosis. Over the years, *Aspergillus* infections have been reported to have features mimicking Tuberculosis. (Franquet *et al.*, 2004; Mwaura *et al*., 2013). *Aspergillus flavus* has been reported to be a human pathogen linked to aspergillosis of the lungs and sometimes causing other infections such as otomycosis and infection of orbital tissues of the eye. It is a regular mold observed to be in the environment and is considered the second typical cause of aspergillosis after *Aspergillus fumigatus*. *Aspergillus fumigatus* is a known saprotrophic fungus that is ubiquitous in nature which are normally found in soil and decomposing organic matter, such as compost piles, where it plays a crucial role in carbon and nitrogen recycling (Geiser, 2009; Mwaura *et al*., 2013).

There is a common belief that *Aspergillus fumigatus* cannot attack living tissues but can only live on preceding lacerations in the lungs because it lacks primary pathogenic effect. Also, studies have revealed that for aspergillosis to occur, the lungs must have been previously damaged by active tuberculosis. Previous reports have shown that *Aspergillus niger* is unlikely to cause disease in human compared to other *Aspergillus* species, but aspergillosis can occur if these fungal spores are inhaled in large amounts. (Ryan and Ray, 2004; Mwaura *et al*. 2013)

Conclusion

This study has established that fungi are significant pathogens found in the respiratory tract of infected patients. It has also shown *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus* to be among the most predominant fungi that can cause respiratory tract infection. As reported by Chowdhary *et al.* (2016), *Aspergillus* species can cause respiratory infection in patients that have tested negative to Tuberculosis.

The fungal species identified in this study is proof of mold-causing infection and it has helped in establishing that *Aspergillus* species are responsible for the respiratory infection in patients whose sputum were analyzed. It is known that with most filamentous fungal spores when inhaled in small or large amounts in this case that of *Aspergillus*, immune-compromised individuals are susceptible to *Aspergillus* infections causing invasive aspergillosis in most cases. It is possible that individuals in the population encountered in this study were exposed to fungal spores or have been treated for Tuberculosis at some point in their lives.

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