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Occurrence and Distribution of *Aspergillus* species among presumptive Tuberculosis Patient in Otukpo Local Government Area of Benue State, Nigeria

*[1]Odagboyi, Godwin, [1]Gberikon, Grace Mwuese, [1]Yaji, Mnena Eunice, and [1]Iwodi, Cornelius

[1]Departmemt of Microbiology, Faculty of Science, Joseph Sarwuan Tarka University, Makurdi, Nigeria.

Correspondence:godwinodagboyi@gmail.com; +2348032899172

Abstract

Aspergilloma is often misdiagnosed as tuberculosis (TB) in developing countries where the prevalence of TB is high, hemoptysis is often equated with TB, and most patients are diagnosed clinically. This study aims to determine the Occurrence and Distribution of Aspergillus species among presumptive tuberculosis patients in Otukpo Local Government Area of Benue State, Nigeria. A total of 461 clients gave their consent and were involved in this research work from four (4) different healthcare facilities in the Otukpo Local government area. The samples were packaged in triple nylon, stored in a cold chain box, and were taken to the general hospital Otukpo laboratory and/or St Charles Hospital Adoka laboratory for analysis. Each Sputum sample was divided into two parts. One part was analyzed for Tuberculosis using PCR (GeneXpert Machine) and the second part was inoculated on Sabouraud Dextrose Agar Medium for identification of Aspergillus species. The Aspergillus species isolated were identified using cultural and comparative pictorial characteristics. Exactly 216 patients had an Aspergillus species prevalence rate of 46.9 %, and 245 patients with 53.1% showed no growths of fungal. The males had a prevalence rate of 113(44.8 %), with the highest rate of 27(10.7 %) in the age group 31 - 40, and females had a prevalence rate of 103(49.3 %), with the highest rate of 18(8.6 %) in the age groups 21 - 30 and 31 - 40 with a significant p-value of 0.015. Co-infections of Aspergillus species/tuberculosis in relation to age range distribution and gender had a total prevalence of 22(4.77 %). The males had a co-infection prevalence rate of 14(3.03%) compared to the females having a co-infection prevalence rate of (1.74%). The age range of 31- 40 had the highest co-infection prevalence rate, followed by the age range of 41-50. There is a significant co-infection of Aspergillus/tuberculosis with a p-value of 0.007. Four species of Aspergillus were identified; Aspergillus niger (38 %) ranked highest, followed by Aspergillus fumigatus (25 %), Aspergillus flavus (24 %), Aspergillus oryzae (6 %), and other fungi isolated were 7%. This research shows a significant positive result of patients with aspergillosis, which is a significant co-morbidity in pulmonary tuberculosis patients in Otukpo Local Government Area of Benue State; this could be misdiagnosed as relapse or treatment failures in the absence of reliable diagnostic and clinical management. The progress of the disease and prolonged treatment with antibiotics or immunosuppressive agents make TB patients susceptible to fungal infection. Patients with persistent clinical symptoms despite TB treatment should be subjected to fungal investigations before re-treatment. Aspergillosis is also one of the nosocomial infections, so patients and healthcare givers should be encouraged to always go for routine fungal infection investigation if few of the symptoms are experienced.

Keywords: Aspergillus, Tuberculosis, Otukpo Local Government, GeneXpert,

INTRODUCTION

Aspergillus species are a type of conidial fungi or asexual fungi. However, it is known that some of them are Ascomycota teleomorphs or sexual beings. All *Aspergillus* species are members of the phylum Ascomycota based on DNA evidence (Samson *et al.*, 2014). The genus's members may grow in environments with high osmotic pressure, such as those with high concentrations of salt, sugar, etc. Due to the high oxygen tension, *Aspergillus* species—which are extremely aerobic, are found in nearly all oxygen-rich habitats. In these settings, they often grow as molds on the surface of substrates. Fungi typically thrive on carbon-rich substrates such as polysaccharides and monosaccharides. (Sotirios *et al.*, 2024) Often, *Aspergillus* organisms contaminate starchy foods.

Several species of fungi also grow on carbon sources. *Aspergillus* species are a family of mold that may grow in or on a variety of plants and trees, including bread and potatoes (Lisa, 2022).

Aspergillus has oligotrophy, the ability to grow in conditions that are completely devoid of essential nutrients or that are nutrient-depleted. One good example of this is *Aspergillus niger,* which is a common component of mildew that grows on damp walls. Many *Aspergillus* species, such as *Aspergillus fumigatus* and *Aspergillus niger,* are known to spread quickly throughout buildings, especially in warm, humid places like restrooms and the spaces surrounding windows. (Mousavi *et al.*, 2016).

Numerous clinical syndromes are caused by *Aspergillus* species, Conidia infection through inhalation is frequent and is based on the host's level of immunological impairment (Chris and David, 2015). While it's evident that fungal infections, including those caused by *Aspergillus fumigatus,* are becoming more common worldwide, it's unclear how big of an issue this is exactly (Zhang *et al.*, 2023).

Numerous illnesses, including Human Immunodeficiency Virus, Acquired immunodeficiency Disease Syndrome, Tuberculosis, Chronic Obstructive Pulmonary disease, the increasing use of immunosuppressive treatments, and many others, have been linked to this increase (Oladele *et al.*, 2017). As a result of tuberculosis (TB), an estimated 1.2 million persons worldwide suffer from Chronic Pulmonary Aspergillosis (CPA), with Southeast Asia, Africa, and the Western Pacific regions being the most afflicted (Ofori *et al.*, 2016).

Due to the fact that hemoptysis is frequently mistaken for tuberculosis and other differential diagnoses, it can be difficult to detect *Aspergillus* disease in developing nations where tuberculosis is highly prevalent. Aspergilloma's natural history is still mainly unknown (Ofori *et al.*, 2016). Nonetheless, Aspergilloma occurs in fewer than 10 percent of the patients (Ali *et al.*, 2021). Aspergillosis and tuberculosis frequently cause the following signs and symptoms: fever, chest pain, coughing up blood, trouble breathing, and weight loss (CDC, 2021).
The actual disease burden of Aspergilloma in sub-Saharan Africa, Nigeria, as well as its impact on morbidity, is unknown (Oladele and Denning, 2014). Aspergillomas are often misdiagnosed as tuberculosis (TB) in developing countries where the prevalence of TB is high, hemoptysis is often equated with TB, and most patients are diagnosed clinically. It is clear that the global burden of fungal infections such as those caused by *Aspergillus* spp is increasing, but the exact magnitude of the problem remains unknown (Lisa, 2022).

The burden of serious fungal infections has not been properly described in Nigeria. despite these substantial underlying disease frequencies. There is very little epidemiological data on serious fungal infections in Nigeria; only a few case reports of invasive fungal infections are available to our knowledge. Therefore, estimating the burden of serious fungal infections in Nigeria based on the populations at risk to provide useful data to highlight the burden of the diseases in our country (Nigeria) and for comparison with data from other parts of the world is very important (Oladele and Denning, 2014).

The findings of this study would help to provide an effective means of identification for treatment of aspergillosis among presumptive tuberculosis patients, which would bring relief from the burden of this infection.

MATERIALS AND METHODS

Study area

Four designated health facilities, General Hospital Otukpo, Comprehensive Health Center, St Daniel Hospital, and St Charles Hospital Adoka, situated in Otukpo Local Government Area, Benue State, were involved in the study. Otukpo LGA has a GPS of Latitude 7° 11' 53.57"N and Longitude of 8° 8' 21.54"E with an average humidity of 61% and a UV-index of 7. The climate is tropical in Otukpo. The average annual temperature in Otukpo is 27.2 °C. In August, the average temperature is 25.5 °C.

Study population

The study Population are patients suspected of having Tuberculosis

Ethical Approval

The Benue State Ministry of Health's Ethical Clearance Committee was contacted in order to get ethical approval, and it was given with the Reference number Ref. MOH/OFF340/VOL4/P8924. A consent form attesting to their willingness to participate in the study was issued to patients and patient caregivers. All data collected throughout the study were kept confidential.

Inclusion/ Exclusion criteria
All patients who were willing to participate and who were suspected of having tuberculosis infection were included in the study; those who were not suspected of having tuberculosis infection and did not consent to the study were excluded.

Study Sample
Sputum samples totaling 461 were obtained from the consented suspected TB patients. The formula shown below was used to calculate the sample size according to Raosoft (2014) sample size calculator using a study population of 199,009. A single population proportion formula was used to calculate the sample size:

n= Z2p (1-p)/d2
Where:
n = Number of samples to be use
Z = The standard normal distribution at 95 % confidence interval=1.96
d = degree of accuracy desired (tolerable error) =5 % =0.05
P=Prevalence (Prevalence of 50 % will be considered).

The sample size was calculated as 392, but a total of 461 patients from the four healthcare facilities were gathered to determine the ratio of patient attendance in each facility.

Sample collection
Four hundred and sixty-one (461) sterile plastic leak-proof containers were distributed to the consented participants, and they were informed on how to provide the sputum samples. Three milliliters (3 mL) of sputum were collected and packaged in triple nylon inside a cold chain box and transported to the laboratories of General Hospital Otukpo and/or St Charles Hospital Laboratory for tuberculosis analysis using the GeneXpert method (WHO, 2013) and for culturing on Sabouraud Dextrose Agar plate. This was done over the period of one year.

Sample Inoculation
Each sample was smeared on a prepared SDA culture plate using a sterile wire loop and sealed with masking tape. The inoculated SDA plates were then incubated at room temperature inside a cupboard and checked every 24hrs for Fungal growth for up to seven (7) days before being discarded as negative, as described by Ochei and Kolhatkkar (2019).

Tuberculosis Diagnosis Using the GeneXpert Machine
After homogenizing the Sputum with a transfer pipette and adding twice the volume of Xpert MTB/RIF Sample Reagent (2 ml to 1 ml) within the leak-proof plastic container, the Sputum was violently shaken 15 times and vortexed for at least 10 seconds. Following the violently shaking of the sample fifteen times and vortexed for at least ten seconds, the mixture was incubated for a further ten minutes at room temperature. This incubation period lasted for five more minutes. Two milliliters of the processed sample were transferred using a brand-new transfer pipette to the Xpert MTB/RIF cartridge. The cartridge was then put into the GeneXpert device in accordance with the manufacturer's instructions for analysis and result generation (Shatha, 2020).

Data Analysis
The data generated were subjected to Chi-square Statistical Analysis and into a worksheet of Statistical Package for Social Science (SPSS) software 20.0. Frequency and percentage were analyzed as descriptive findings. $P<0.05$ was accepted as statistically significant.

RESULTS

Occurrence of *Aspergillus* species
Out of the 461 samples analyzed, 216 (46.9 %) were positive for *Aspergillus* species, while 245 (53.1 %) showed no growth. St Daniel Hospital Otukpo had the highest percentage prevalence rate of 55.7 % Followed by St Charles Hospital Adoka having 53.9 %), then CHC Otukpo 46.3%, and the least is GH Otukpo with a percentage prevalence rate of 41.8 % shown in Table 1 below. The differences in the prevalence in the selected health facility was statistically insignificant (x^2; P=0.462) at 0.05 confidence level.

Table 1: Percentage Occurrence of *Aspergillus* species in the Four Health Facilities in Otukpo LGA

Facility Distribution (n)		No Positive (%)	Prevalence %	No Negative (%)
GH Otukpo	244	102 (47.2)	41.8	142(58)
CHC Otukpo	54	25 (11.6)	46.3	29(11.8)
SDH Otukpo	61	34 (15.7)	55.7	27 (11.0)
SC Adoka	102	55(25.5)	53.9	47 (19.2)
TOTAL	**461**	**216**	**46.9**	**245**

Key: x^2(3df) = 2.58, P=0.462, P>0.05 (No association) x^2 ; df = 3 = 2.58, P=0.462, p>0.05

Table 2: Percentage occurrence of *Aspergillus* species in Relation to Age Range and Gender

Age (Years)	Number Studied	No of Males	No Positive (%)	No of Females	No Positive (%)	P -Value
0 - 10	38	19	3 (2.6)	19	8 (7.8)	0.015
11 - 20	52	27	14 (12.4)	25	16 (15.5)	
21 - 30	77	42	18 (15.9)	35	18 (17.5)	
31 - 40	101	61	27 (23.9)	40	18 (17.5)	
41 - 50	82	40	22 (19.5)	42	17 (16.5)	
51 - 60	54	26	13 (11.5)	28	16 (15.5)	
61 above	57	37	16 (14.2)	20	10 (9.7)	
Total	**461**	**252**	**113 (44.8%)**	**209**	**103(49.3%)**	

The result of the study in Table 2 shows the occurrence of *Aspergillus* species in relation to age and gender with a significant p-value of 0.015. In males, the highest occurrence rate is 27 (23.9 %) in the age group of 31 - 40, followed by 22(19.5 %) in the age group 41 - 50, and the lowest is (0 - 10) with 3(2.6 %). For the female, the highest prevalence rate are 18(17.5%) in age groups 21 - 40, followed by 17(16.5 %) for 41 - 50. and the least is 0 - 10 with 8(7.8%).

Table 3: Occurrence of *Aspergillus* species in patients having TB as Co-Infection in Relation to Age Range Distribution and Gender

Age (Year)	Number studied	Total positive (% Prevalence)	No of Males (% Prevalence)	No of Females (% Prevalence)
0 -10	38	0 (0)	0 (0)	0 (0)
11 - 20	52	1 (4.5)	0 (0)	1 (12.5)
21 - 30	77	4 (18.2)	2 (14.3)	2 (25)
31 - 40	101	6 (27.3)	5 (35.3)	1 (12.5)
40 - 50	87	5 (22.7)	4 (28.6)	1 (12.5)
51 - 60	54	4 (18.2)	3 (21.4)	1 (12.5)
61 Above	57	2 (9.1)	0 (0)	2 (25)
Total	**461**	**22 (4.77%)**	**14 (3.04%)**	**8 (1.73%)**

C-infection and Age: $x^2(6df) = 41.83$, $P=0.000$, $P<0.05$ (Association exists)
Co-infection and age: $x^2(1df) = 7.398$, $P=0.007$, $P<0.05$ (Association exists).

The result of the study in Table 3 shows the occurrence of *Aspergillus* species and tuberculosis co-infections in relation to age range distribution and gender, with a total occurrence of 22 (4.77 %) of *Aspergillus* /tuberculosis co-infections. The males had a more prevalence rate of 14(3.03%) compared to the females having a co-infection prevalence rate of (1.74%). The age range of 31- 40 had the highest Co-infection prevalence rate, followed by the age range of 41-50. There is a significant co-infection of p-Value 0.000 and 0.007.

Table 4: Percentage distribution of *Aspergillus* species growth

Isolate	Frequency (%)
Aspergillus Fumigatus	54 (25)
Aspergillus flavus	52 (24)
Apergillus niger	82 (38)
Aspergillus oryzae	13 (6)
Other Fungi	15 (7)
Total	**216**

Out of the 216 positive samples, *Aspergillus niger* (38 %) ranked highest, followed by *Aspergillus fumigatus* (25 %), *Aspergillus flavus* (24 %), *Aspergillus oryzae* (6 %), and other fungi isolated were 7% (Table 4).

DISCUSSION

Aspergillosis is a disease of medical concern, the findings of this study revealed a high rate of aspergillosis (46.8 %) across the four health facilities studied. This is similar to the results of Maduakor *et al.* (2020) study, which shows a prevalence of 42.6 %. This investigation also aligned with the Japanese study conducted by Tashiro *et al.* (2011), which recovered 165 species of *Aspergillus* from 139 patients.

They reported that 62 (45 %) people had no aspergillosis symptoms at all but were colonized with *Aspergillus* species, and 77 (55 %) people had pulmonary aspergillosis that was classified as either chronic necrotizing pulmonary aspergillosis (CNPA) (48 %), aspergilloma (29%), IPA (13 %), or allergic bronchopulmonary Aspergillosis (ABPA) (10 %). A lower prevalence of 24.2 % and 29 %, respectively, was found by Maiz *et al.* (2015) and Shrimali *et al.* (2013). The high relative humidity of the Otukpo Local Government Area's environment may be the cause of the high prevalence. Together with other fungi, four species of *Aspergillus* were isolated in the current study. *Aspergillus niger* (38 %) ranked highest, followed by *Aspergillus fumigatus* (25 %), *Aspergillus flavus* (24 %), *Aspergillus oryzae* (6 %), and other fungi isolated ware 7%, in contrast to the result of Maduakor *et al.,* (2020), whose study indicates that *Aspergillus fumigatus* had the highest isolation rate of 36.1 % followed by *Aspergillus niger* (28.4 %), but it differs from the findings of Amiri *et al.* (2016), who found that *Aspergillus flavus* was the most common fungus in tuberculosis patients' sputum. *Aspergillus flavus* ranked third (24%), the prevalence of this mold in the environment, Otukpo has been dusty, and it is a serious risk to people with a compromised immune system or underlying diseases. The disease is known to occur in sporadic and epidemic form and has public health and economic implications (Pal *et al.*, 2014). Several studies have shown varying rates of isolation; these variations may be the result of various diagnostic standards, racial disparities, living situations, and geographical climates (Amiri *et al.*, 2016). The occurrence of *Aspergillus* species and tuberculosis co-infections in relation to age range distribution and gender had a total of 22 (4.77 %). The males had *Aspergillus*/tuberculosis co-infection rate of 14 (3.03 %) compared to the females with 8 (1.74 %). This is almost similar to Moatharesadat *et al.* (2020), who found that *Aspergillus* and tuberculosis co-infection varied between 3.7 % and 33.3 %, the co-infection is varied between 0.2 % and 1.3 %. Contrary to Reza *et al.* (2018), who reported that the age ranges 41- 50 years had the highest *Aspergillus*/tuberculosis co-infection, this study shows that the age range of 31- 40 had the highest co-infection prevalence rate followed by the age range of 41-50. There is a significant co-infection of p-Value 0.007. Although a widely accepted approach has been put in place to combat tuberculosis (TB), the illness still poses a serious threat to public health.

CONCLUSION

According to this study, there are significant occurrences of *Aspergillus* species, which are distributed among categories of patients, both males and females, with more occurrence among the working force age groups. This is a challenge as it can affect the economic growth of Otukpo LGA. Pulmonary Aspergillosis is also a significant co-infection in pulmonary tuberculosis patients in Otukpo LGA, Benue State. In the absence of accurate diagnostic and clinical management, these co-existing diseases could be misdiagnosed as relapse or treatment failure. From this study, females are more infected with *Aspergillus* species than males. Four species of *Aspergillus* were isolated with other fungi. *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus oryzae*, and other fungi. Despite the widespread adoption of a strategy to control tuberculosis (TB), a notable percentage of patients have tuberculosis and or co-infections with *Aspergillus* and tuberculosis.

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