 ISSN 2955-1226 (Print)
ISSN 2955-1234 (Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

Available online at https://www.tjpps.org

Original Research Article

Molecular Epidemiology of *Aspergillus* Species Isolated From Individuals With Suspected Case of Pulmonary Tuberculosis In Adamawa State, Nigeria

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ABSRTACT

Aspergillus is an opportunistic pathogen with varying disease diversity, and those with underlying issues are more likely to become infected. In sub-Saharan Africa, it is among the pathogens reported from people with respiratory illness in the early case series and negative acid-fast bacillus sputum smears. This study aimed at the molecular epidemiology of *aspergillus* species isolated from individuals with suspected cases of pulmonary tuberculosis in Adamawa, Nigeria. A total of 425 early morning sputum from patients attending seven selected tuberculosis clinics using a standard guideline were collected. The specimens underwent bacteriological, mycological, and molecular analysis for species characterization and antifungal susceptibility patterns. The prevalence of fungal species among presumptive tuberculosis patients was 39.8%, and males (57.4%) were significantly affected. The risk of infection is significantly related to older age and habits such as smoking and alcoholism. *A. flavus* is the highest of the fungi recovered at 7.51%, followed by *A. niger* (4.23%). Itraconazole, voriconazole, and caspofungin expressed excellent in vitro activity against the twelve species tested, and amphotericin B showed 3% resistance to non-wild type *A. flavus* and *A. fumigatus*. This outcome can represent epidemiological data to provide insights into the prevalence and susceptibility profiles of the pathogens. The species identification of the *Aspergillus* in the region provided accurate and relevant information to guide treatment decisions and develop strategies to prevent the emergence of resistance.

Keywords: Molecular, *Aspergillus*, Pulmonary tuberculosis, antifungal and susceptibility testing

Received 01 April 2024
Revised 03 May 2024
Accepted 30 May 2024
Published online 01 July 2024

Introduction

The global mortality rate of fungi-related disease is 1.5M of the estimated proportion of people that come down with the disease. Chronic pulmonary *aspergillosis* is a gradual destructive lung disease on continuous exposure to aspergillus species. It accounts for about 0.5M deaths among 3M people who suffer from the disease.[1] The recent global estimate reported 1.2M CPA as a consequence of previous pulmonary tuberculosis (PTB) and most of the cases occur in WHO's Southeast Asia, Western Pacific, and African regions, where pulmonary tuberculosis has the highest prevalence.[2]

World Health Organization (WHO) global 2020, reported 10.4 million incident cases of tuberculosis (TB) worldwide. However, Nigeria is one of the 30 high-burden countries for TB, TB/ HIV, and DR-TB.[3] The estimated incidence of tuberculosis in Nigeria is 219 per 100000 populations with only 15% of the total burden of the disease in the country being notified in 2015.[4] One in fifteen tuberculosis cases typically progresses to chronic pulmonary aspergillosis, potentially complicating treated pulmonary tuberculosis. In high tuberculosis prevalence areas, the diagnostic criteria for chronic pulmonary aspergillosis and pulmonary tuberculosis are so similar that differentiation without proper diagnosis is challenging.

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Citation: Yusuf, AA, Kumurya, AS, Yusha'u, M. Molecular Epidemiology of *Aspergillus* Species Isolated from Individuals with Suspected Case of pulmonary tuberculosis in Adamawa State, Nigeria. Trop J Phytochem Pharm. Sci. 2024; 3(3): 222-229
http://www.doi.org/10.26538/tjpps/v3i3.2

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Without tests, patients are frequently misdiagnosed with conditions like 'smear-negative pulmonary tuberculosis,' 'progressive upper lobe fibrosis,' or 'recurrent pulmonary tuberculosis,' leading to inappropriate or lack of therapy.[5] *Aspergillus fumigatus*, has been mostly documented in pulmonary aspergillosis, rare cases are caused by *A. niger*, *A. nidulans*, and *A. flavus*. However, if diagnostic tests are not conducted, aspergillosis becomes underdiagnosed.

There are many different types of pulmonary and extra-pulmonary diseases related to TB, which might have serious problems in differential and therapeutic diagnosis.[6] These cases might also be aggravating factors associated with chronic obstructive pulmonary diseases, diabetes, elderly patients, high cholesterol levels, lung cancer, immunodeficiency, and fungal lung infections. In recent times, there has been a strong interest in fungal infection diagnosis due to the fact that patients presenting fungal diseases have presented several lung infections such as TB. The frequency of these infections and the number of immunosuppressive disease cases have gradually increased.[6]

Pulmonary infections are often missed because it is associated with underlying diseases with no specific clinical manifestations and increased rates of morbidity and mortality. Fungi infection has emerged as a worldwide healthcare problem in the last decade due to widely used broad-spectrum antibiotics and steroids.[7] It can be acquired primarily or secondarily in tuberculosis (TB), immunodeficiency patients, and other chronic diseases such as diabetes mellitus or malignancy may worsen the primary disease.[8]

The burden and mortality associated with these diseases have not been given deserved attention in underdeveloped and LMIC countries. Thus, early drug susceptibility testing of the available antifungal agents is essential for successful treatment and assessment of possible drug resistance. The major antifungals used in the clinical setting to treat fungal infections are azoles, polyenes, and echinocandins represented by voriconazole, amphotericin B, and caspofungin.[9]

In Nigeria, it would be desirable if pulmonary aspergillosis receives more public health attention with more resources allocated from the government for its surveillance, physical facilities, outbreak response, epidemiological study, and control. In this research study, the molecular epidemiology of *Aspergillus* species in Adamawa state was examined through phenotypic and genotypic approaches to indicate the relevance of *Aspergillus* screening for presumptive tuberculosis patients. This will serve as an algorithm for the diagnosis and proper management of tuberculosis that may be complicated with aspergillosis.

Objectives of the study are as follows:

- To assess the frequency of *Aspergillus* infection among presumptive tuberculosis patients in Adamawa state.
- To isolate, identify, and characterize *Aspergillus* species among patients in the rural and urban settlements of Adamawa state.
- To determine the pattern and distribution of *Aspergillus* infection concerning age and gender among the tuberculosis suspects in Adamawa state.
- To determine the susceptibility of *Aspergillus* species to some common antifungal drugs in Adamawa State.

Materials and Methods

Study Area and Population

Adamawa State is located in the savannah belt of the North Eastern part of Nigeria and lies between Latitude 90 141 N of the equator and Longitude 120 281 E of the Greenwich meridian. Adamawa State shares boundaries with Taraba State in the South and West, Gombe State in its Northwest, and Borno State to the North. Adamawa State has an international boundary with the Republic of Cameroon along its eastern border. It covers a land area of about 38,741 km^2. It has a population of 3,168,101.[10]

The DOT centers were employed to serve as a means of accessing the patients and the study covered at least seven local government areas out of the twenty-one in the state. The LGAs are Mubi South, Mubi North, Girei, Yola South, Yola North, Numan, and Ganye to cover at least two from each senatorial district. The study population includes patients of all ages and sexes enrolled in DOTs with presumptive cases of pulmonary tuberculosis disease under the National Tuberculosis and Leprosy Control Programme (NTBLCP).

Inclusion and Exclusion Criteria

All presumptive tuberculosis patients who presented at the clinic and were referred to the laboratory for AFB were enrolled in the study. Thus, any patients on antifungal drugs or HIV positive were excluded from the research.

Study Design

The study was cross-sectional and hospital-based, and the consecutive sampling method was used because it is a non-probability type that gives room to convenient sampling of the targeted participants.

Sample Size Determination and Collection

The minimum sample size of 358 was determined using a standard formula of Araoye.[11] A total of four hundred and twenty-five (425) early morning sputum samples were collected from TB suspects who consented as described by USAID.[12]

Direct Microscopy and AFB for ZN Staining

The sputum prepared with 10% KOH was examined with the magnification ×10, then ×40 Objective (CX 21 Olympus microscope, model CX22RFS1, Tokyo, Japan) for the presence of fungal hyphal fragments. Prepared labeled slides were stained by the ZN method for AFB identification.[13]

Culture-based identification

The samples for fungal culture were inoculated into Sabouraud glucose agar for genus identification, supplemented with chloramphenicol (0.5 mg/ml) (SC), and incubated at 30°C for 4 weeks.[14] The *Aspergillus* species were identified by subculturing on a meat extract agar (MEA) plate. Inoculated MEA were incubated reverse side up at 30°C, with additional MEA plates incubated at 37°C for characteristics identification. Colony-forming structures of mold isolates obtained by slide culture on SDA followed by lactophenol cotton blue staining were examined microscopically for primary identification at the genus/species level.

Further identification of *Candida* was done by inoculation on CHROMagar Candida (CHROMagar Microbiology, Paris, France), incubated at 35 °C for 2 days and observed for specific colony colors: *C. albicans* Light-Green, *C. tropicalis* Blue, *C. Krusei* Pale pink, *C. parapsilosis* Pink, *C. glabrata* Pale pink, *C. lusitaniae* Pink, *C. nivariensis* Cream to white, and *C. guilliermondii* Light Pink according to the instructions of the manufacturer.[14]

Antifungal Sensitivity Testing

In-vitro antifungal susceptibility testing was performed according to the CLSI M38-A2 micro broth dilution method for filamentous fungi (Clinical and Laboratory Standards Institute.[15] The antifungal agents tested are amphotericin B (AMB), voriconazole (VRC), itraconazole (ITC), and caspofungin (CAS) (Sigma-Aldrich, St. Louis, MO, USA). All antifungal drugs were tested in concentrations ranging from 0.006 to 16 µg/ml. *Pichia kudriavzevii* (*Candida krusei*) (ATCC 6258) was used as a quality control (QC) strain as indicated in CLSI M38-A2.

Molecular Analysis

Molecular approaches are crucial to the accurate identification of *Aspergillus* species because of their similarity and overlapped morphological features as the traditional (morphology) way is not reliable. The ITS (internal transcribed spacer) and βt (beta-tubulin) universal primers for *Candida* and *Aspergillus* species were respectively adapted from the work of Abdel-Azeem *et al*.[14]

DNA extraction of Candida and Aspergillus

DNA of yeast isolates were extracted using the boiling method as described by Abdel-Azeem *et al*.[14] Meanwhile, phenol-chloroform techniques described by Aboutalebian *et al*. were used for *Aspergillus* species.[16]

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR – RFLP) Analysis

Species identification of Candida isolates was confirmed by the internal transcribed spacer (ITS) PCR-RFLP method using the MspI restriction enzyme.[17] For the isolates of *Aspergillus*, a fragment of the β-tubulin gene was amplified and sequenced using bt2a and bt2b primers.[14]

Statistical analysis

The data was analyzed using statistical software (SPSS version 25.0). Categorical analysis was performed using the Chi-square tool (x2) in SPSS, and differences among means were determined using ANOVA. Descriptive analytical tools such as frequency count, percentage, and prevalence were determined using MS Excel in Office 2010. The significant levels were considered to be less than 0.05 ($P< 0.05$).

Ethics consideration

Ethical approval was obtained from the Ethical Committee of Adamawa State Ministry of Health (Ref. No: S/MoH/1131/1) and Federal Medical Centre, Yola (Ref. No: FMCY/SUB/S.128/79). A copy of the informed consent form was given and/or read to each participant to sign or thumbprint for his/her acceptance to participate in the study. Then, the questionnaire was administered to consented participants.

Results and Discussion

Distribution of Fungi Species among Patients with Suspected Cases of Tuberculosis in Adamawa State

The results in Figure 1 show the overall distribution of fungi species among patients with suspected cases of tuberculosis in Adamawa State. Thus, irrespective of location, *A. flavus* is the highest fungi with 7.51%, followed by *A. niger* with overall proportion of 4.23% others are less than one percent (<1%) proportionate respectively. However, *C. albicans*, *Penicillium* spp, *Mucor* spp and *C. tropicalis* shared significant proportionate of 5.87%, 5.16%, 2.82% and 2.58% respectively.

Table 1 presents the outcomes of isolation and characterization of *Aspergillus* species among patients with suspected cases of tuberculosis in the rural and urban settlements of Adamawa State. As shown in the Table 1, among TB patients, the urban settlers recorded higher *A. flavus* (16.13%) followed by *A. niger* (9.68%), while among rural settlers the highest fungi species was *Penicillium* spp (10.34%) followed by *C. albicans* (6.90%) and *C. glabrata* (6.90%). In overall, irrespective of settlement, patient with tuberculosis recorded higher *A. flavus* (10.00%), followed by *A. niger* (6.78%), *C. albicans* (6.78%) and *Penicillium* spp (6.78%). Also, the non-tuberculosis patients settled in urban area recorded higher *A. flavus* (8.52%) followed by *C. albicans* (4.93%) and *Penicillium* spp (4.48%), while among the AFB negative patient settled in rural area, the higher fungi recorded was *C. albicans* (6.99%), followed by *Penicillium* spp (5.59%) and *A. flavus* (4.90%).
In overall, irrespective of settlement the AFB negative patient recorded higher *A. flavus* (7.10%) followed by *C. albicans* (5.74%) and *Penicillium* spp (4.92%).
Aspergillus is among the most abundant and widely distributed fungal species on earth, adapting to a variety of habitats and niches such as air, water, soil, plant debris, decaying vegetation, saw dust litter, leaf litter, animal feed, organic compost piles, and other similar environment. The most common transmission route for aspergillosis is inhalation of airborne fungal spores. In some cases, *Aspergillus* can also cause infections in people with compromised skin, such as those with cuts or wounds, as the fungus can enter through broken skin.
The precipitation patterns, humidity, temperature, and wind conditions are factors in the risks of exposure, which vary both temporally and geographically.[14] Exposure of people who have undergone invasive procedures, such as organ transplantation or implantation of medical devices to a*spergillus* spores can cause infections. It is among the pathogens reported in early case series of people with respiratory illness and negative acid-fast bacillus (AFB) sputum smears in sub-Saharan Africa.[5] They are an important cause of life-threatening infection in immunocompromised patients and an occasional cause of morbidity among healthy individuals such as *Aspergillus* osteomyelitis where about 34% of patients have no obvious predisposing factor or immunosuppression. Such individuals may be infected through direct inoculation of traumatized skin; contiguous spread from pleuro-pulmonary disease; or hematogenous spread.[8]

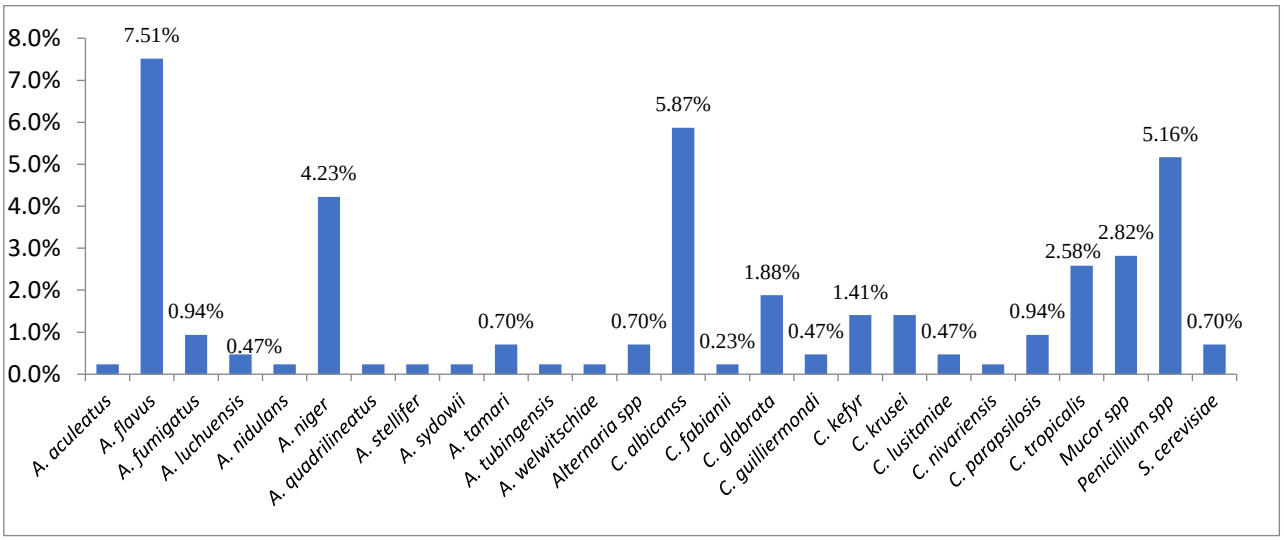

Figure 1: Distribution of Fungi Species among Patients with Suspected Cases of Tuberculosis in Adamawa State

Table 1: Distribution of Fungi Species among Patients with Positive and Negative Cases of Tuberculosis in Adamawa Sta

Isolates	Tuberculosis (n=59)			Non- tuberculosis (n=366)		
	Urban	Rural	Overall	Urban	Rural	Overall
A. aculeatus	1(3.23)	0(0.0)	1(1.67)	0(0.0)	0(0.0)	0(0.0)
A. flavus	5(16.13)	1(3.45)	6(10.0)	19(8.52)	7(4.9)	26(7.1)
A. fumigatus	0(0.0)	0(0.0)	0(0.0)	2(0.9)	2(1.4)	4(1.09)
A. luchuensis	0(0.0)	0(0.0)	0(0.0)	1(0.45)	1(0.7)	2(0.55)
A. nidulans	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)	1(0.27)
A. niger	3(9.68)	1(3.45)	4(6.67)	9(4.04)	5(3.5)	14(3.83)
A. quadrilineatus	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)	1(0.27)
A. stellatus	0(0.0)	1(3.45)	1(1.67)	0(0.0)	0(0.0)	0(0.0)
A. sydowii	0(0.0)	0(0.0)	0(0.0)	1(0.45)	0(0.0)	1(0.27)
A. tamarii	0(0.0)	0(0.0)	0(0.0)	3(1.35)	0(0.0)	3(0.82)

A. tubingensis	0(0.0)	0(0.0)	0(0.0)	1(0.45)	0(0.0)	1(0.27)
A. welwitschiae	0(0.0)	0(0.0)	0(0.0)	1(0.45)	0(0.0)	1(0.27)
Alternaria spp	0(0.0)	0(0.0)	0(0.0)	1(0.45)	2(1.4)	3(0.82)
C. albicans	2(6.45)	2(6.9)	4(6.67)	11(4.93)	10(6.99)	21(5.74)
C. fabianii	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)	1(0.27)
C. glabrata	0(0.0)	2(6.9)	2(3.33)	3(1.35)	3(2.1)	6(1.64)
C. guilliermondi	0(0.0)	0(0.0)	0(0.0)	1(0.45)	1(0.7)	2(0.55)
C. kefyr	0(0.0)	1(3.45)	1(1.67)	4(1.79)	1(0.7)	5(1.37)
C. krusei	0(0.0)	0(0.0)	0(0.0)	3(1.35)	3(2.1)	6(1.64)
C. lusitaniae	0(0.0)	1(3.45)	1(1.67)	0(0.0)	1(0.7)	1(0.27)
C. nivariensis	0(0.0)	0(0.0)	0(0.0)	1(0.45)	0(0.0)	1(0.27)
C. parapsilosis	0(0.0)	0(0.0)	0(0.0)	3(1.35)	1(0.7)	4(1.09)
C. tropicalis	0(0.0)	0(0.0)	0(0.0)	7(3.14)	4(2.8)	11(3.01)
Mucor spp	0(0.0)	0(0.0)	0(0.0)	9(4.04)	3(2.1)	12(3.28)
Penicillium spp	1(3.23)	3(10.34)	4(6.67)	10(4.48)	8(5.59)	18(4.92)
S. cerevisiae	0(0.0)	1(3.45)	1(1.67)	2(0.9)	0(0.0)	2(0.55)
No Fungi Growth	19(61.29)	16(55.17)	35 (58.33)	131(58.74)	88(61.54)	219(59.84)

Table 2: show the percentages of susceptibility, intermediate profile, and resistance against azoles, CAS and AMB considering the breakpoints. Antifungal susceptibility patterns and MIC of Aspergillus spp. using the CLSI METHOD

Species	**Antifungal agent**	**No. of isolates Categorized as**			**Species**	**Antifungal agent**	**No. of isolates Categorized as**		
		S	**I**	**R**			**S**	**I**	**R**
A. fumigatus (4)	Amhotericin B	3	0	1		Amhotericin B	1	0	0
	Itraconazole	4	0	0	*A. welwitschiae* (1)	Itraconazole	1	0	0
	Voriconazole	4	0	0		Voriconazole	1	0	0
	Caspofungin	4	0	0		Caspofungin	1	0	0
A. flavus (32)	Amhotericin B	31	0	1	*A. luchuensis* (3)	Amhotericin B	3	0	0
	Itraconazole	32	0	0		Itraconazole	3	0	0
	Voriconazole	32	1	0		Voriconazole	2	1	0
	Caspofungin	32	0	0		Caspofungin	3	0	0
A. tamarii (3)	Amhotericin B	3	0	0	*A. nidulans* (1)	Amhotericin B	1	0	0
	Itraconazole	3	0	0		Itraconazole	1	0	0
	Voriconazole	3	0	0		Voriconazole	1	0	0
	Caspofungin	3	0	0		Caspofungin	1	0	0
A. niger (18)	Amhotericin B	18	0	0		Amhotericin B	1	0	0
	Itraconazole	18	0	0	*A. quadrilineatus* (1)	Itraconazole	1	0	0
	Voriconazole	18	0	0		Voriconazole	1	0	0
	Caspofungin	18	0	0		Caspofungin	1	0	0
A. aculeatus (1)	Amhotericin B	1	0	0	*A. stellatus* (1)	Amhotericin B	1	0	0
	Itraconazole	1	0	0		Itraconazole	0	0	0
	Voriconazole	1	0	0		Voriconazole	0	1	0

	Caspofungin	1	0	0		Caspofungin	1	0	0
A. tubingensis (2)	Amhotericin B	2	0	0	*A. sydowii* (1)	Amhotericin B	1	0	0
	Itraconazole	2	0	0		Itraconazole	1	0	0
	Voriconazole	1	1	0		Voriconazole	1	0	0
	Caspofungin	2	0	0		Caspofungin	1	0	0
Total (68)	Amhotericin B	66(97%)		2(3%)					

S: Susceptible, I: Intermediate, R: Resistant

In all pulmonary syndromes, *Aspergillus fumigatus* is the most common species agent of infection, followed by *Aspergillus flavus* as a more common cause of various forms of allergic rhino sinusitis, postoperative aspergillosis, and fungal keratitis. Although *Aspergillus terreus* is a common cause of invasive aspergillosis, this has been found occasionally in *Aspergillus niger* that are common colonizer of the respiratory tract.[5]

According to the findings in Table 1, there are higher prevalence of potential mycoses in the urban area than observed among rural settlers. It could be due to favorable factors such as diet, lifestyle, and geography that may substantially shape the spread of fungi agents. It is in line with the finding made from the work by Martins *et al.* [18] Gange and Brown found that in urban centers activities such as diet, lifestyle, and geography are likelihood factors influencing the proportional spreading of fungi.[19] Though, a study by Hughes *et al*. that compared the presence of fungi infection in urban and rural areas found insignificant differences in terms of proportion but concluded that other factors liable to increase or decrease the proportion of fungi infection found in particular settlements can be traced to geographical factors.[20] Also, the high temperatures that are mostly experienced in the urban centers could be amiable factors for breeding and spreading of fungi infection in urban than rural. This fact has been established by Garaga *et al*. that rural communities benefit from natural vegetation which controls or regulates the temperature of the area, compared to an urban settlement that has its space opened to direct sunlight due to a lack of natural vegetation as a result of construction activities, mass settlement, and industrialization.[20] A study by Savić *et al*. found that temperatures between 25 and 30°C allow growing of fungi.[22] More so, the temperature was found to account for fungi growth in warm temperatures region of Serbia. Recent work by Patel *et al*. attributed high fungi growth to very high temperatures of 130-150°F.[23] However, a study by Adams *et al*. showed that some fungi thrived in very low temperatures below 32°F (below freezing).[24]

Other evidence suggesting factors for a higher proportion of fungi in urban centers pointed at climate change, which was linked to the increase in fungal diversity and abundance in urban areas.[11] Thus, the rise in temperature and humidity levels, especially in cities, creates an ideal environment for the growth and survival of fungi. One of the features of urban sentiment is massive growth and development through mass building projects which involves the conversion of rural areas to urban areas. Patel *et al.* linked mass building projects to the increase in fungal diversity in urban areas than observed in rural.[23] This is because urbanization disrupts the natural ecosystems, leading to the introduction of new plant species and habitats that support fungal growth. The buildings in urban areas, such as high-rise buildings, create a conducive environment for the growth and proliferation of fungi.[21]

The spreading of fungi in urban centers was also attributed to increased air pollution.[23] Osherov found that urban areas tend to have higher levels of air pollution, which is responsible for the accumulation of fungal spores in the air and easily travel long distances.[25] Apart from pollution, built-up area, and industrialization, the urban area is characterized by higher population densities than rural areas, which accounted for an increase in human activity and disturbance of natural environments that further release fungal spores into the air.

Table 3: Distribution of Fungal Isolates in relation to Gender and Age of patients

Variable	Number Tested	Negative	Positive	% Positivity per group	Prob.Value
Gender					
Male	269	172	97	57.40	0.002
Female	156	84	72	42.60	
Age group					
<11	30	15	15	8.88	
11 – 20	58	37	21	12.43	
21 – 30	107	70	37	21.89	
31 – 40	94	54	40	23.67	0.011
41 – 50	62	37	25	14.79	
51 – 60	25	18	7	4.14	
60+	49	25	24	14.20	

The disparities in fungi proportion among the presumptive TB patients living in rural and urban areas of Adamawa state as found in this study is not peculiar to only Nigeria, as other foreign works have established significant differences between fungi infection in urban and rural areas; in India, Kathuria *et al*. found that urban areas had a higher concentration of fungal spores in the air than rural areas.[26] Also, in Brazil, Martins *et al*., Oladele *et al*. in Nigeria, Garaga *et al*. in India, and Asano *et al*. in Japan all reported higher concentrations of airborne fungal spores in urban than in rural areas.[18, 2. 21, 27]

It was established in the study by Abdel-Azeem *et al*. that *A. flavus* can attack various tissues including the skin, eye, heart, brain, and joint.[14] However, the tissue damage is more severe in those whose immunity is compromised due to other reasons. The finding made by Hughes *et al*. after a comparison of pathogenicity and clinical syndromes due to *Aspergillus fumigatus* and *Aspergillus flavus* revealed that *A. flavus* is a common cause of fungal sinusitis and cutaneous infections.[20] Thus, it was concluded that the bigger size of *A. flavus* spores, in comparison to those of *A. fumigatus* spores, favoured their deposit in the upper respiratory tract

In Vitro Antifungal Susceptibility of the Tested Antifungal Drugs

Itraconazole and caspofungin revealed the best activities against compared to voriconazole (Table 2). The resistance of 3% were found in amphotericin B on two isolates of *A. fumigatus* and *A. flavus*.

The antifungal susceptibility data generated in this study indicate that itraconazole and caspofungin have excellent in vitro activity against the twelve species tested. In this research, 100% susceptibility was demonstrated by voriconazole and itraconazole. Kathuria *et al*. reported the antifungal susceptibility of 108 *Aspergillus* isolates and found the highest agreement for voriconazole (100%) and amphotericin B (75%) in MICs.[26] In addition to caspofungin (GM 0.008), itraconazole appears more active against isolates of *A. flavus* compared to voriconazole and amphotericin B. This may be supported by the fact that the patient has not been on long-term use of azole therapy and their environment might not have been exposed to agricultural use of fungicides that are the main

route of resistance development.[24] More so that the treatment of fungi infections is of low priority in our community. Similar results are obtained in previous studies

Distribution of Fungal Isolates in relation to Gender and Age of the patients

Table 3 presents the distribution of fungi isolates in relation to gender and age of the patients. The results showed that the rate of isolation of fungi is higher in male 57.40% than female 42.60%. There was statistically significant association between the prevalence of fungal and the sex of patients ($P < 0.05$). In general, the percentage positivity rate per age group depicted those patients in the age group of 21 to 30 and 31 – 40 were slightly more affected than younger age groups. The association of fungal isolation rate and age was statistically significant ($P < 0.050$).

The findings (Table 3) on the distribution of fungal isolates to gender and age of patients show that the fungi isolation rate is higher in males than females. The result from the analysis further revealed a statistically significant association between the prevalence of fungal isolation rate and the sex of patients ($P < 0.05$). The difference in the proportion of males and females with fungi infection could be traced to behavioural variations, as well as differences related to biological sex. This concurs with the findings by Hughes *et al*. that biological sex, which comprises differences in host sex hormone homeostasis and immune responses, has a substantial impact on the epidemiology of infectious diseases.[20] Though, Osherov established that comprehensive data on sex distributions in invasive fungal diseases (IFDs) are lacking.[25] Also, Adams *et al*. reported 51.2% of invasive candidiasis cases, mostly matching the proportions of females among the general population in the United States, while in Europe >51% was recorded.[24] In contrast, Kathuria *et al*. found that other IFDs were overrepresented in males, including invasive aspergillosis (51% males), mucormycosis (60%), cryptococcosis (74%), coccidioidomycosis (70%), histoplasmosis (61%) and blastomycosis (66%). This shows that the gender of an individual could lead to varying degrees of exposure to various fungi diseases.[26]

There is still a need for further investigations concerning the association between biological sex/gender and the pathogenesis of IFDs are warranted. The association of fungal isolation rate and age was statistically significant in the study by Morley, with more findings among males than females. Also, Osherov found that the host sex or gender influences the incidence of some fungal infections in humans such as aspergillosis, cryptococcosis, paracoccidioidomycosis, dermatophytosis, and candidiasis.[25 28]

The beta-tubulin marker has been previously used to study the phylogeny within the *Aspergillus* genus and other related species because it is a conserved, slow-evolving gene with a high degree of interspecific variability in the intronic regions. In this study, the beta-tubulin gene was used to classify the *Aspergillus* spp isolated from presumptive TB patients, corroborating its value as a phylogenetic marker for species identification.[29]

Conclusion

Reports on the phenotypic identification of *Aspergillus* in this region are scarce, genotypic analysis was used for identification at the species level. Therefore, identifying *Aspergillus* isolates at the species level through molecular methods represents a significant impact, since these may be associated with allergic conditions. The molecular epidemiology of *Aspergillus* species in Adamawa State revealed that the prevalence of fungi among presumptive tuberculosis patients is high, and males are significantly affected. The risk of being infected is statistically related to old age, alcohol, and smoking habits of an individual. The beta tubulin-based assessment assisted in typing with explicit identification of the fungal pathogens and incidence. It is relevant since these findings contribute to fungal biodiversity and ecology.

There is a need to consider repeated isolation of the identical *Aspergillus* species and detection of anti-Aspergillus antibodies and/ or *Aspergillus* circulating antigens in the sera of presumptive TB patients. It will validate the report that the isolated species represents the etiological organism in immunocompetent or mildly immunocompromised individuals.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

I hereby appreciate the support of Prof M. T. Hedayati and Prof S. H Mirhendi who facilitated the elaboration and realization of this work.

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