

Prevalence of Fungal Infection among Presumptive Tuberculosis Patients

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ABSTRACT

Tuberculosis (TB) remains a significant global health concern, particularly in resource-constrained settings like Nigeria. Co-infections with fungi further complicate TB diagnosis and management, often leading to misdiagnosis and suboptimal treatment outcomes. Fungal infections, which mimic TB symptoms, are common among immunocompromised individuals, particularly those with presumptive TB. This cross-sectional study aimed to determine the prevalence and spectrum of fungal infections among 350 presumptive TB patients attending the Directly Observed Therapy (DOT) clinic of the University of Port Harcourt Teaching Hospital, Nigeria. Sputum samples were collected and analyzed for fungal and TB pathogens using culture, molecular methods, and microscopy. Fungal species were identified through standard mycological techniques and molecular characterization. Data on socio-demographic characteristics and clinical history were obtained through questionnaires and standard laboratory request forms. Statistical analyses were performed using descriptive and inferential statistics, with a significance level of $p < 0.05$. The general prevalence of fungal infections was 64.3%, with 225 participants testing positive. Molecular identification revealed *Candida albicans* as the most prevalent fungal pathogen (61.8%), followed by *Aspergillus udagawae* (11.1%) and *Aureobasidium pullulans* (8.9%). Co-infection of TB and fungi was observed in 8.0% of the participants. Males constituted 61.7% of the study population, and the age group 21–30 years had the highest representation (32.3%). Marital status analysis showed a higher prevalence of single participants (57.7%). The high prevalence of fungal infections among presumptive TB patients highlights a critical need for integrated diagnostic and treatment strategies targeting fungal co-infections. *Candida albicans* and *Aspergillus udagawae* were the predominant pathogens identified. Routine fungal screening for TB patients, improved diagnostic facilities, and antifungal therapies should be incorporated into TB management programs. Further studies focusing on antifungal resistance and clinical outcomes of co-infected patients are recommended.

Keywords: *Candida albicans*, *Aspergillus udagawae*, fungal infections, presumptive tuberculosis, tuberculosis co-infection.

Introduction

Tuberculosis (TB) remains a significant public health challenge, especially in low-income countries, where it accounts for substantial morbidity and mortality (Darma et al., 2020). *Mycobacterium tuberculosis*, the causative agent of TB, predominantly affects the lungs, manifesting as pulmonary tuberculosis (PTB). Despite progress in TB diagnosis and treatment, co-infections, particularly fungal infections, have become a growing concern (Darma et al., 2020).

Fungal infections, or mycoses, are diseases caused by pathogenic fungi, many of which affect the respiratory system. Pulmonary fungal infections may either mimic TB symptoms or coexist with it, complicating diagnosis and treatment.

This overlap is particularly critical in presumptive TB patients presenting with clinical and radiological signs of TB yet to be confirmed microbiologically.

Mycoses occur on a variety of body sites such as skin, soft tissues, blood and lungs. Tuberculosis, on the other hand, is predominantly in the lungs. These groups of infections have been neglected over the years despite their high prevalence in humans. Pathogenic fungi are major causes of health challenges, yet are given less attention in the diagnosis of pulmonary infections. The current study focuses on pulmonary mycoses as tuberculosis also affect the respiratory system. Although the true burden of fungal respiratory tract infection is elusive, the frequency of the infection has been increasing in the last few decades (Fauci & Morens, 2012).

Pulmonary tuberculosis (PTB), HIV/AIDS, chronic obstructive pulmonary disease, and extensive use of immunosuppressive drugs are incriminated for such an increase (Chakrabarti, 2005).

Pulmonary fungal infection (pulmonary mycosis) is an infectious disease of the lungs that is caused by fungi. The infection develops after the colonization of the lungs by fungi or their spores through inhalation, or the reactivation of latent infection, or via haematogenous dissemination. Fungi or their spores are abundant and exist virtually everywhere in the human environment (Bitew & Bati, 2021).

Given the ubiquitous nature of fungi and their spores, colonization or infection of the lungs is unavoidable, but mechanisms of differentiating fungal colonization from fungal infection are not well-established hence, the subject remains a serious challenge. Isolation of fungi alone or in association with *M. tuberculosis* from presumptive tuberculosis patients (i.e., patients presenting signs and symptoms of pulmonary tuberculosis and having radiological characteristics of pulmonary tuberculosis) was considered as fungal infection or pulmonary mycosis (Bitew & Bati, 2021). Many fungal species have been reported as etiological agents of lung infection. In most literatures, species of *Aspergillus* (Yao & Liao, 2006), *Candida*, *Cryptococcus* (Kronstad et al., 2011), *Pneumocystis* (Udwadia et al., 2004), and thermally dimorphic fungi (Lortholary et al., 1999) are the most significant. However, the epidemiology of fungi causing lung infection has been changing. Accordingly, many mycelial fungi, such as *Scedosporium* spp., *Fusarium* spp., *Penicillium* spp., dematiaceous filamentous fungi, zygomycetes, and yeasts other than *Candida albicans*, have emerged as etiological agents of respiratory disorders. While these fungi are rarely recovered in the respiratory tract of immune-competent individuals, they may disseminate to other systemic organs producing life-threatening invasive fungal diseases in individuals already experiencing serious illnesses (Nucci & Marr, 2005).

Globally, fungal infections have been increasingly recognized as a serious health threat, with reports of their rising incidence, particularly among immunocompromised individuals. Factors such as HIV/AIDS, chronic TB treatment, prolonged corticosteroid use, and other forms of immunosuppression exacerbate the vulnerability to fungal infections in these patients (Dzhurabaeva et al., 2021).

However, fungal infections remain underdiagnosed in resource-constrained settings, such as Nigeria, where TB is endemic (Dzhurabaeva et al., 2021). The clinical and radiological similarities between TB and pulmonary fungal infections often lead to misdiagnosis, resulting in empirical TB treatment with poor outcomes for fungal infections (Dzhurabaeva et al., 2021).

The rising prevalence of fungal infections and their potential to complicate TB diagnosis and treatment highlight the importance of this study. While TB remains a focus of public health research, the burden of fungal infections in presumptive TB patients is often overlooked. This study seeks to address the knowledge gap by providing data on the prevalence of fungal infections among this group in Nigeria. Understanding the prevalence and spectrum of fungal pathogens in presumptive TB cases will inform diagnostic and therapeutic strategies, reducing misdiagnoses and improving patient outcomes. Furthermore, the study will contribute to the development of evidence-based policies for managing co-infections and addressing antifungal resistance.

This study aims to determine the prevalence of fungal infections among presumptive TB patients attending the University of Port Harcourt Teaching Hospital. This study will address the significant knowledge gap in understanding fungal infections among presumptive TB patients. First, the study seeks to isolate and identify fungal species present in the sputum samples of individuals suspected of having tuberculosis (TB).

Materials and Methods

Study Design

This study used a cross-sectional design and laboratory investigation involving three hundred and fifty presumptive tuberculosis patients attending the Directly Observed Therapy (Dot) clinic of University of Port Harcourt Teaching Hospital. The study used a well-structured questionnaire to obtain socio demographic data of the study subjects. Some data of the participants were also obtained from a standard laboratory request form completed by physicians. The presumptive tuberculosis patients sputum were collected for microscopy and culture for mycosis while tuberculosis assessment was done using microscopy, GeneXpert and culture methods.

The samples were transported promptly to the mycology laboratory unit of UPTH according to the standard operating procedures for collection, biosafety and shipment of infectious material (United Nation, 2019).

The population of this study was patients seeking health service at the Dot clinic of the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State.

Study Area

This study was carried out at University of Port Harcourt Teaching Hospital in the neighbourhood of Port Harcourt, the capital of Rivers state, southern Nigeria. A tertiary health care facility and a referral hospital. It lies along the Bonny River, 41 miles (66 kilometer) upstream from the Gulf of Guinea, and is located in the Niger Delta with a metro area population of 3,325,000. Subjects were recruited from the Dot clinic in University of Port Harcourt Teaching Hospital (UPTH) in Obio/Akpo LGA.

Sample Size

The minimum sample size of this study was determined based on a single population proportion formula. The prevalence of co-infection of pulmonary mycoses recorded in 2020 was 34.4% (Amala *et al.*, 2020). Using this prevalence in the standard equation: $n = \frac{ZxZxP(1-p)}{dx}$, where n = sample size, z = 95% statistic for level of confidence (1.96), P = population proportion (34.4% or 0.344), and d = margin of error (degree of accuracy desired (d = 0.05)). This gives a minimum sample size of 350 people.

Inclusion Criteria

Patients with provisional diagnosis of tuberculosis, particularly those with a persistent cough for more than three weeks and can produce sputum at the time of sample collection were recruited in the study. Only patients who consented to participate in the study with evidence of signed written consent were included.

Exclusion Criteria

Apparently, healthy individuals with no clinical symptoms of pulmonary infection, and those who are under antifungal or tuberculosis treatment were excluded from the study.

Ethical Consideration

The study ethical approval was issued by the University of Port Harcourt Teaching Hospital ethical committee. Written consent was obtained for all patients and personal information was handled with utmost confidentiality.

Sampling Methods

A convenient sampling method was utilized to achieve the estimated sample size. All pulmonary tuberculosis presumptive patients attending Dot Clinic in the University of Port Harcourt Teaching Hospital, Rivers State within the period of study were included in the study. The demographics of the study included sampling of equal male and female participants.

Microbiological Analysis of Samples

The microbiological analysis involved detecting Mycobacterium tuberculosis (MTB) and fungal isolates from sputum samples through culture, microscopy, molecular techniques, and other diagnostic methods. MTB detection utilized Lowenstein-Jensen (LJ) medium for culturing, which inhibits contaminants while promoting early growth of mycobacteria. Sputum samples underwent decontamination using NALC-NaOH, followed by inoculation on LJ medium and incubation at 37°C for eight weeks, with weekly monitoring for growth (Cadmus *et al.*, 2011).

Confirmation of MTB isolates employed the SD Biline TB Ag MPT64 assay, which identified MTB complex from Acid-Fast Bacilli-positive cultures. Additionally, microscopy using Ziehl-Neelsen staining provided a rapid, cost-effective method for detecting acid-fast bacilli, while the GeneXpert MTB/RIF assay offered precise molecular diagnosis, including rifampicin resistance detection (Fred, 2009)

For fungal analysis, sputum samples were examined using potassium hydroxide (KOH) preparation and inoculated on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol. The SDA plates were incubated at 37°C and room temperature for 2-4 weeks to allow for fungal growth. Lactophenol Cotton Blue staining and colony morphology helped identify moulds and yeasts, with germ tube tests confirming *Candida* species.

Molecular characterization of fungal isolates involved DNA extraction using ZR fungal/bacterial DNA kits, followed by quantification with a Nanodrop spectrophotometer to ensure DNA quality for downstream applications. These combined methods provided comprehensive microbiological insights into the samples (Javadi *et al.*, 2014).

Data Analysis

In the statistical analysis of the data collected, descriptive statistics were employed, with results expressed as percentages. The Chi-square test was utilized to ascertain the association between the rates of infection and various socio-demographic variables.

Throughout the analysis, the level of significance was established at α of 0.05, indicating that results with a p-value less than 0.05 would be considered statistically significant.

Results

Table 1 presents the distribution of socio-demographic variables among the study participants (N=350). Sex is categorized into Male and Female, with 61.7% males and 38.3% females. Age is categorized into age groups: <21, 21-30, 31-40, 41-50, 51-60, and 61 and above, with respective percentages of 15.4%, 32.3%, 28.3%, 13.7%, 8.6%, and 1.7%. Marital Status includes Single (57.7%) and Married (42.3%) participants.

Table 2 illustrates the general prevalence of fungi infection among the study participants (N=350) and Fungi infection positive (225, 64.3%) and negative (125, 35.7%). And also the prevalence of Tuberculosis (TB) and Fungi Coinfection among the study participants (N=350). The variable is classified into Positive and Negative categories, with 28 participants (8.0%) testing positive for TB & Fungi Coinfection and 322 participants (92%) testing negative. The total percentage accounts for 100.0% of the study population.

Table 1: Frequency Distribution of socio-demographic variables of Study Participants

Variable	Classification	Frequency (N=350)	Percent
Gender	Male	216	61.7
	Female	134	38.3
	Total	350	100.0
Age (Years)	<21	54	15.4
	21-30	113	32.3
	31-40	99	28.3
	41-50	48	13.7
	51-60	30	8.6
	61 and above	6	1.7
	Total	350	100.0
Marital Status	Single	202	57.7
	Married	148	42.3

Table 2: General Prevalence of Fungi Infection in study population

Variable	Classification	Frequency (N=350)	Prevalence Rate
Fungi Infection	Positive	225	64.3%
	Negative	125	35.7%
TB & Fungi co-infection	Positive	28	8.0
	Negative	322	92.0
	Total	350	100.0

Table 3 illustrates the distribution of fungal infections by molecular identification among the participants in the study, with a total sample size of 350 individuals. The table includes the following fungal species: *Curvularia caricae-papayae.*, *Pseudopithomyces chartarum.*, *Aspergillus udagawae.*, *Penicillium sp.*,

Trichoderma longibrachiatum., *Candida albicans.*, *Cutaneotrichosporon curvatum.*, *Trametes polyzona.*, and *Aureobasidium pullulans.* For each species, the table lists both the number of cases and their corresponding prevalence percentage

Table 3: Prevalence of Fungi Infections in Study Participants

Species	Frequency (N=350)	Prevalence Rate (%)
<i>Curvularia caricae-papayae</i>	6	2.7
<i>Pseudopithomyces chartarum</i>	5	2.2
<i>Aspergillus udagawae</i>	25	11.1
<i>Penicillium sp.</i>	12	5.3
<i>Trichoderma longibrachiatum</i>	8	3.6
<i>Candida albicans</i>	139	61.8
<i>Cutaneotrichosporon curvatum</i>	1	0.4
<i>Trametes polyzona</i>	9	4.0
<i>Aureobasidium pullulans</i>	20	8.9

Figure 1 below shows the association between gender and fungal prevalence. Among the participants, males have a higher frequency (138) and percentage (61.3%) of fungal prevalence compared to females (87, 38.7%). The P-value of 0.91 indicates no statistically significant association between gender and fungal prevalence.

Figure 2 displays the association between age and fungal prevalence. The highest prevalence is observed in the 21-30 age group (67, 29.8%), followed by the 31-40 age group (63, 28%). Prevalence decreases progressively in older age groups, with the lowest frequency in those above 60 years (6, 2.7%). The P-value of 0.03 indicates a statistically significant association between age and fungal prevalence.

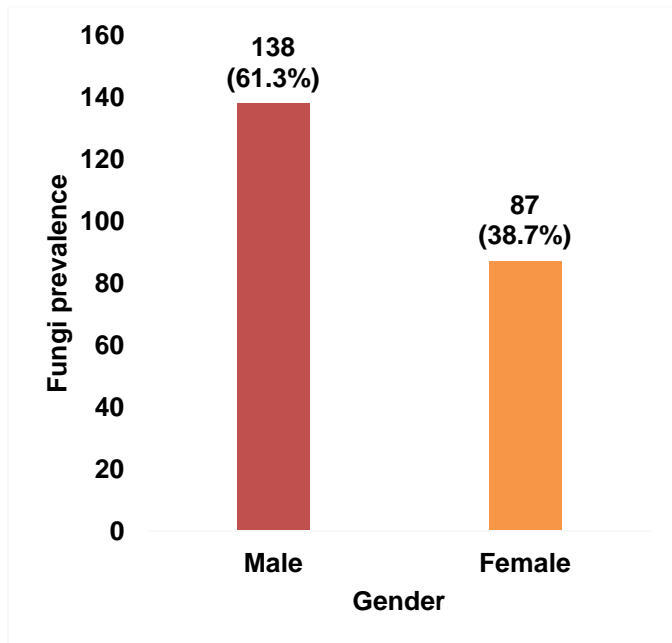


Fig. 1: Association between Fungal infection prevalence and Gender

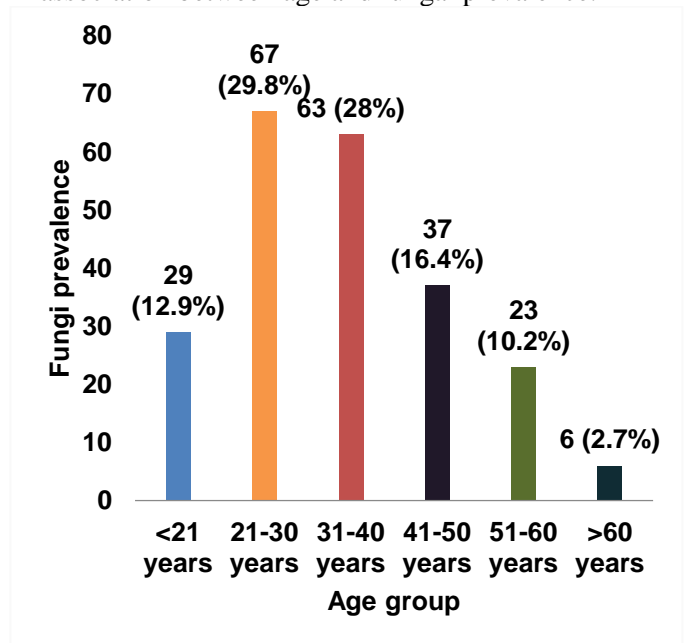


Fig. 2: Association between Fungal infection prevalence and Age

Figure 3 shows the association between marital status and fungal prevalence. Married individuals have a slightly higher frequency (121) and percentage (53.6%) of fungal prevalence compared to single individuals (104, 46.4%). The P-value of 0.23 indicates no statistically significant association between marital status and fungal prevalence.

Figure 4 shows the bar chart which displays the chi-square test statistics for various comparisons involving Fungi Isolation against other diagnostic tests (Sputum

AFB Staining, TB Isolation, Confirmatory TB Test, and GeneXpert). Each bar height represents the chi-square value for the corresponding test comparison, with p-values displayed above the bars.

All tests (e.g., Fungi Isolation vs. TB Isolation and Fungi Isolation vs. Sputum AFB) show relatively low chi-square values (ranging from 2.11 to 2.81), indicating weak association between Fungi Isolation and other diagnostic methods.

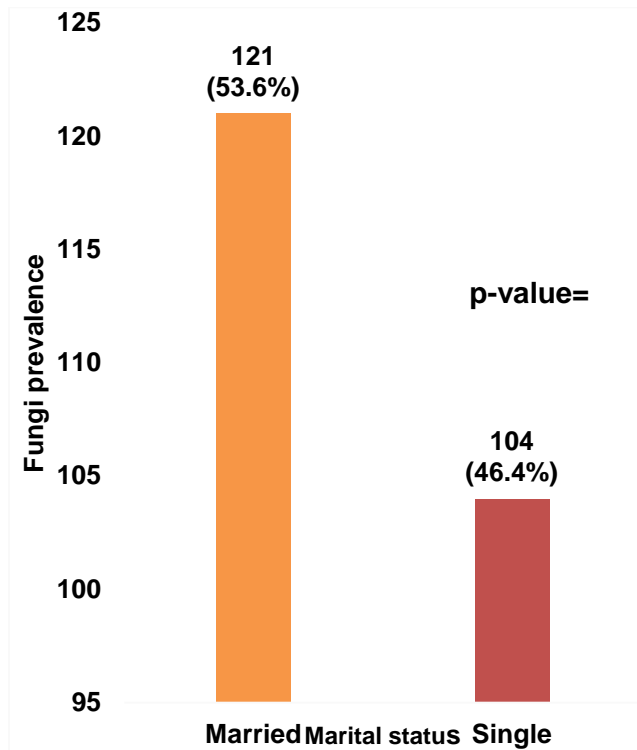


Fig. 3: Association between Fungal Infection Prevalence and Marital Status

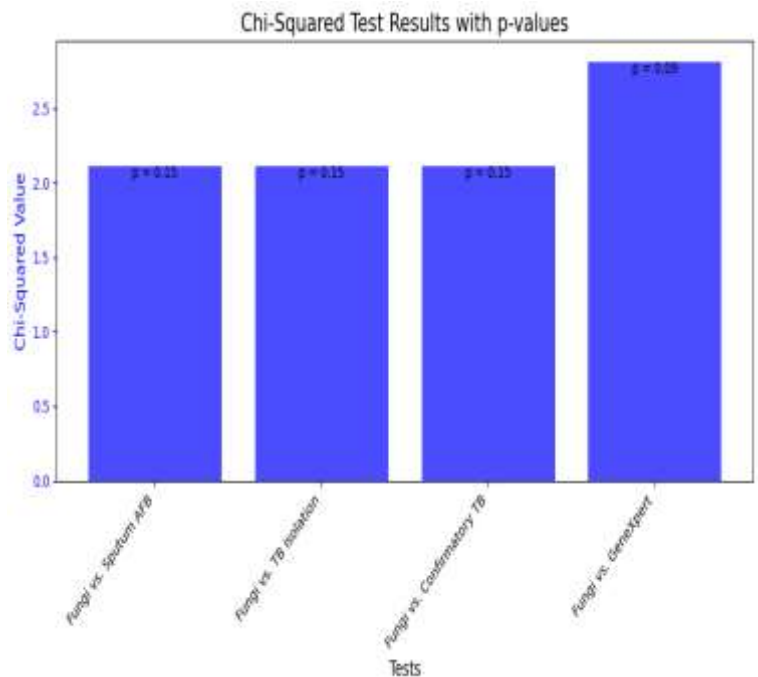


Fig. 4: Comparison of Fungal Infection and Different Diagnostic Techniques

Discussion

One significant finding from the study is that the majority of the presumed tuberculosis patients were males (61.7%). This is in line with multiple earlier studies (Sulis et al., 2019; WHO, 2020) that found that men were more likely than women to contract tuberculosis. Numerous factors, such as variations in healthcare-seeking behaviour, occupational exposures, and hormonal influences on immunity, have been linked to men's higher susceptibility to tuberculosis (Garcia-Garcia et al., 2019).

In addition, males may be more exposed due to societal roles and behaviors, particularly in places where they are more likely to work in environments that can spread tuberculosis, like mines, construction sites, and crowded urban areas (Bates et al., 2019). It's important to remember, though, that there may be regional differences in the distribution of genders, influenced by socioeconomic, healthcare, and cultural norms. The study's presumed tuberculosis patients' age distribution reveals a peak in the 21–30 age range, with older age categories showing a gradual decline after that.

This pattern aligns with the global tuberculosis epidemiology, which frequently affects young adults disproportionately (WHO, 2020). Emerging views that young adults are more likely to participate in activities that raise their exposure to risk, such as living in communal settings like dorms or shared housing and interacting with people in large crowds. This study is in consonance with Jasmer *et al.* (2019). Furthermore, younger people might be more socially mobile, which raises the likelihood that they will encounter TB-infected people. Nonetheless, given that age-related immune decline and comorbidities increase susceptibility to severe forms of tuberculosis, it is important to pay attention to the study population's lower representation of people of age 61 and above (Alagna *et al.*, 2019). Other reasons for discrepancy are lower healthcare-seeking behavior, decreased diagnostic sensitivity, and underreporting of tuberculosis cases among older adults.

The marital status distribution of presumed tuberculosis patients shows a greater percentage of single people (57.7%) than married people (42.3%). Although marital status alone may not directly affect a person's risk for tuberculosis, it can be a good indicator of underlying socioeconomic factors and patterns of healthcare utilization. The relationship between marital status and tuberculosis risk has been the subject of conflicting findings in the past (Kliiman *et al.*, 2019; Li *et al.*, 2021). For example, some research has found that the prevalence of tuberculosis is higher in single people, and it has been linked to variables like social isolation, higher rates of migration, and a lower socioeconomic status (Kliiman *et al.*, 2019). On the other hand, after controlling confounding variables, some research has not found a significant relationship between the incidence of tuberculosis and marital status (Li *et al.*, 2021). Consequently, although marital status can function as a stand-in for some social determinants of health, its actual influence on the prevalence of tuberculosis may differ based on contextual elements like cultural norms, healthcare accessibility, and population demographics. The findings on the general prevalence of fungal infection indicate that fungal infections are a significant health concern within the study population, with a prevalence rate of 64.3% among participants.

This aligns with global trends showing a rising burden of fungal infections, particularly in regions with limited healthcare resources, high HIV prevalence, or

a significant burden of tuberculosis (TB) (Bongomin *et al.*, 2019; Zhu *et al.*, 2021). The high prevalence observed in this study reinforces the critical need for enhanced diagnostic and treatment strategies to address fungal diseases effectively.

Several studies have reported similar high prevalence rates of fungal infections in populations at risk. For instance, Zhu *et al.* (2021) conducted a meta-analysis showing elevated rates of invasive fungal diseases among individuals with compromised immune systems, such as TB or HIV co-infection (Zhu *et al.*, 2021). In such populations, fungal infections often go undetected due to overlapping clinical symptoms with other opportunistic infections (Nacher *et al.*, 2019; Zhu *et al.*, 2021).

The relatively high proportion of participants testing positive for fungal infections (64.3%) might also reflect an underlying immunocompromised status or environmental factors conducive to fungal growth, such as high humidity and poor ventilation (Bongomin *et al.*, 2019; Teixeira *et al.*, 2018). Previous research has documented that *Candida* spp. and *Aspergillus* spp. are the most frequently isolated pathogens in immunosuppressed individuals, especially in developing countries (Bongomin *et al.*, 2019; Waikhom *et al.*, 2020). In our study the high prevalence rate is consistent with findings in similar settings (Nacher *et al.*, 2019; Waikhom *et al.*, 2020).

On the other hand, the negative prevalence rate of 35.7% could represent individuals with robust immune systems or minimal exposure to risk factors. Studies such as those by Bongomin *et al.* (2019) have highlighted that individuals with adequate innate and adaptive immune responses are better equipped to fend off fungal infections (Bongomin *et al.*, 2019; Teixeira *et al.*, 2018). Moreover, the role of routine antifungal prophylaxis in at-risk populations may explain the lower prevalence in some subsets of the population (Bongomin *et al.*, 2019; Torosantucci *et al.*, 2009). According to the results of TB and fungi coinfection based on molecular identification, 8.0% of the presumptive tuberculosis patients tested positive for both TB and fungi coinfection, whereas 92.0% tested negative. The findings emphasize how crucial it is to take fungal coinfections into account when treating patients with presumed tuberculosis, as these coinfections can impede the effectiveness of diagnosis and treatment.

Immunocompromised individuals, including TB patients, are frequently diagnosed with fungal infections, especially those caused by opportunistic pathogens like *Aspergillus*, *Candida*, and *Cryptococcus species*. (Bongomin et al., 2019). Integrated management strategies that address both TB and fungal coinfections are necessary to improve patient outcomes, as indicated by the prevalence of TB and fungi coinfection found in this study.

When comparing these results to earlier research, several studies have found the prevalence of fungal infections among TB patients to be either higher or similar. For instance, in a high-burden TB setting, a study by Nacher et al. (2019) reported a prevalence of 70% for fungal coinfections among TB patients. Similarly, a study conducted in a different geographic region by Zhu et al. (2021) found that 58.6% of TB patients had fungal infections. These results support the notion that fungal coinfections are frequent in tuberculosis patients in a variety of contexts, given the prevalence of TB and fungi coinfection seen in the current investigation.

It is crucial to remember, however, that prevalence rates of fungal infections in TB patients can differ based on several factors, including location, demographics of the population, and underlying comorbidities. Variations in the diagnostic techniques employed to identify fungal infections may also be the source of discrepancies among studies. While some studies use molecular or culture-based methods to detect the growth of fungi, others rely on antigen detection assays or clinical diagnosis, which can result in varying estimates of prevalence (Armstrong-James et al., 2020). Furthermore, differences in patient populations, such as disparities in HIV prevalence or the use of immunosuppressive therapy, may impact the likelihood of fungal coinfections in tuberculosis patients.

In Port Harcourt, the species-specific prevalence of fungal infection based on molecular identification among patients suspected of having tuberculosis (TB) offers important information about the range of fungal pathogens that this population is exposed to. A wide spectrum of pathogens can be derived from the distribution of fungal species, the most common of which are *Candida albicans*, *Aspergillus udagawae*, and *Aureobasidium pullulans*.

The prevalence of *Candida albicans* (61.8%) is consistent with other research showing that *Candida* is one of the most prevalent fungal pathogens in tuberculosis patients (Zhu et al., 2021). According to Armstrong-James et al. (2020), *Candida spp.* are opportunistic pathogens that are frequently detected in immunocompromised people, such as TB patients. They can cause a variety of infections, such as invasive candidiasis, esophagitis, and oral thrush. The high prevalence of *Candida albicans* found in this study emphasizes how crucial it is to take fungal coinfections in TB patients into account and administer the proper antifungal therapy when necessary.

Aspergillus udagawae accounted for 11.1% of the fungal infections identified, making it the second most common fungal pathogen. This result is in line with earlier research that showed *Aspergillus species* to be prevalent opportunistic infections in tuberculosis patients, especially in those with lung involvement (Zhu et al., 2020). According to Bongomin et al. (2019), *Aspergillus species* can cause a variety of diseases, from invasive aspergillosis to allergic bronchopulmonary aspergillosis (ABPA), which can make it difficult to diagnose and treat tuberculosis patients. The results of the study regarding the prevalence of *Aspergillus udagawae* highlight the significance of taking *Aspergillus* coinfections into account when treating tuberculosis patients, especially those who exhibit respiratory symptoms or radiological findings that point to fungal involvement.

Aureobasidium pullulans, with a prevalence of 8.9%, was also among the more common fungal pathogens identified in this study. This finding is important as *Aureobasidium pullulans*, although not as widely recognized as *Candida* and *Aspergillus*, can still pose a significant threat to immunocompromised patients.

In addition, species such as *Penicillium sp.* (5.3%), *Trametes polyzona* (4.0%), *Trichoderma longibrachiatum* (3.6%), *Curvularia caricae-papayae* (2.7%), *Pseudopithomyces chartarum* (2.2%), and *Cutaneotrichosporon curvatum* (0.4%) were identified in the study population. Although less common than *Aspergillus udagawae* and *Candida albicans*, these fungal pathogens can still significantly increase morbidity and mortality in patients with impaired immune systems, such as tuberculosis patients (Bongomin et al., 2019).

The presence of multiple fungal species emphasizes the complexity of fungal coinfections in tuberculosis patients and the significance of thorough diagnostic procedures and specialized antifungal therapy.

Similar distributions of fungal species among TB patients have been reported in several studies. For instance, *Candida spp.*, *Aspergillus spp.*, and *Penicillium spp.* were found to be the most prevalent fungal pathogens in tuberculosis patients by Zhu et al.'s meta-analysis in 2021. These results confirm the prevalence of these fungal species found in this study, showing a consistent range of fungal coinfections in TB patients across various geographic locations.

Within the study population, the association between the prevalence of fungal infections and demographic factor data like age, gender, and marital status sheds light on the epidemiology and risk factors of fungal infections.

There was no statistically significant difference between the prevalence of fungal infections and gender at $p < 0.05$. The prevalence of fungal infections was comparable in males (61.3%) and females (38.7%). This result disagrees with some earlier research that found differences in the prevalence of fungal infections between genders (Benedict *et al.*, 2021). Different hormones, immune responses, and environmental exposures can all have an impact on gender differences in the prevalence of fungal infections. The lack of statistical significance in this study, however, raises the possibility that gender is not a reliable indicator of the prevalence of fungal infections in this specific population.

A statistically significant difference was found between the age groups in the association between the prevalence of fungal infection and age at $p < 0.05$ (p -value = 0.03). The age group with the highest prevalence of fungal infection was 21–30 years (29.8%), followed by 31–40 years (28%), and 41–50 years (16.4%). These results are consistent with some earlier research that found differences in the prevalence of fungal infections according to age (Choi *et al.*, 2020). Different age groups may have different prevalence rates of fungal infections due to underlying comorbidities, lifestyle choices, and immune system aging. Given the significant association between age and the prevalence of fungal infections, it is possible that age plays a major role in determining the risk of fungal infections in this population.

There was no statistically significant difference between the prevalence of fungal infection and marital status at $p < 0.05$ (p -value = 0.23). The prevalence of fungal infections was similar in single individuals (46.4%) and married individuals (53.6%). These results run in opposition to some earlier research that found links between the prevalence of fungal infections and marital status (Gugnani *et al.*, 2019). They opined that marital status could affect the risk of fungal infection due to factors like intimate contact, shared living spaces, and household crowding, according to Gugnani *et al.* (2019). The lack of statistical significance in this association, however, raises the possibility that fungal infection prevalence in this specific population is not significantly predicted by marital status.

The matrix of correlation between fungi and diagnostic techniques shows a very strong correlation between Sputum AFB, TB Isolation, and Confirmatory TB (all have a correlation of 1.00, with a p -value of 0.001). This indicates perfect agreement or near-perfect correlation between these tests, suggesting they are likely measuring similar or related phenomena. GeneXpert is also strongly correlated with Sputum AFB, TB Isolation, and Confirmatory TB, with correlation coefficients of 0.97 and p -values of 0.001.

This suggests that GeneXpert aligns well with traditional diagnostic methods, confirming its validity as a diagnostic tool for TB. Fungi Isolation shows no significant correlation with other tests (p -values ranging from 0.09 to 0.15), indicating it operates independently from the TB diagnostic markers. This makes sense as it targets fungal infections rather than TB. It further supports the fact that these tests are measuring different pathogens or clinical conditions.

All chi-squared values are approximately 2.0 or higher, but the p -values for Fungi vs. Sputum AFB, Fungi vs. TB Isolation, and Fungi vs. Confirmatory TB are 0.15. This indicates no statistically significant difference between the fungi test results and these TB diagnostics, suggesting that fungal and TB diagnostics may not be strongly related in these cases. This finding supports the correlation result previously discussed. The comparison between Fungi Isolation and GeneXpert shows a p -value of 0.09, which is closer to the threshold of significance ($p < 0.05$). Although this is not statistically significant, it may indicate a marginal association worth further investigation.

However, the p-value of 0.09 for Fungi vs. GeneXpert could warrant additional exploration in future studies, as it might indicate a weak or emerging pattern. The lack of significant results across the comparisons ($p > 0.05$) suggests that the Fungi Isolation test does not significantly differ from or relate to TB diagnostic tests. This reinforces the distinct nature of fungal infections versus TB.

In conclusion, the findings of this study highlight the significant prevalence of fungal infections among presumptive tuberculosis (TB) patients in Port Harcourt, Nigeria, with a general prevalence rate of 64.3% and co-infection with TB observed in 8% of the study population. *Candida albicans* emerged as the most frequently identified fungal species, followed by *Aspergillus udagawae* and *Aureobasidium pullulans*, indicating a diverse spectrum of fungal pathogens affecting this population. These results underscore the critical need for routine fungal screening in presumptive TB cases to improve diagnostic accuracy and therapeutic outcomes. The overlap in clinical presentations between TB and fungal infections necessitates an integrated diagnostic approach, leveraging molecular techniques to identify fungal pathogens effectively. Public health interventions, including enhanced diagnostic capacity, antifungal therapy protocols, and awareness campaigns, are imperative to mitigate the burden of fungal co-infections and their impact on TB treatment outcomes. This study contributes valuable data to the understanding of fungal-TB co-infections and serves as a basis for future research and policy development.

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