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Isolation of *Cryptococcus* Species from Sputum of Patients with Presumptive Pulmonary Tuberculosis Attending a Referral Hospital in Cross River State, Nigeria

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ABSTRACT

Introduction: The differential diagnosis of pulmonary tuberculosis (TB) and fungal infections is important in influencing treatment strategies and enhancing therapeutic success. Therefore, this study aimed to investigate the prevalence of *Cryptococcus* species among presumptive tuberculosis patients in a referral hospital in Nigeria. **Methods:** This cross-sectional, hospital-based study was carried out between October 2024 and January 2025. A total of 174 sputum samples were tested for the presence of *Mycobacterium tuberculosis* using Ziehl-Neelsen staining and the molecular GeneXpert MTB/RIF assay; *Cryptococcus* species were isolated by culture on Sabouraud Dextrose Agar (SDA) and presumptively identified as encapsulated yeasts using India ink staining. **Results:** None of the samples (0%) obtained from presumptive tuberculosis patients were positive for *M. tuberculosis*, highlighting the importance of investigating other pathogens, such as *Cryptococcus* species, in patients presenting with TB-like symptoms. *Cryptococcus* species were isolated from 21 of 174 patients, giving an overall prevalence of 12.1%. A significant gender disparity was observed, with females showing a much higher prevalence (35.2%) compared with males (1.7%). Age was not significantly associated with infection status, although individuals aged 50–59 years had the highest age-specific prevalence, with 4 out of 20 patients (20.0%) testing positive. Statistical analysis confirmed the significant gender difference ($\chi^2 = 39.426$, $P < 0.001$), while the association with age was not statistically significant (Fisher–Freeman–Halton exact test statistic = 7.681, $P = 0.199$). No *Cryptococcus* isolates were recovered from participants aged 1–9 years, 40–49 years, and ≥ 60 years. **Conclusion:** The moderate prevalence of *Cryptococcus* species observed in this study suggests their potential as a lower respiratory tract pathogen in presumptive tuberculosis patients. The results of this study show the need to integrate fungal screening into routine diagnostic protocols for tuberculosis, especially where fungi such as *Cryptococcus* species are often neglected.

INTRODUCTION

The World Health Organization fungal priority pathogens list (WHO FPPL) is the first global effort to systematically prioritize fungal pathogens, considering their unmet research and development (R&D) needs and perceived public health importance [1]. The global burden of fungal diseases has increased in recent years, with invasive fungal infections (IFIs) emerging as a major cause of morbidity and mortality. This increase is largely driven by the growing population of immunocompromised individuals, the rising prevalence of chronic diseases, and environmental factors such as climate change. At the same time, challenges in early detection, including underdiagnosis, frequent misdiagnosis, and limited availability of reliable diagnostic modalities, contribute to the underestimation and mismanagement of IFIs worldwide, particularly in low- and middle-income countries. Recent global estimates indicate that approximately 6.5 million cases of IFIs occur annually, resulting in an estimated 3.8 million deaths [2].

The growing global burden of fungal diseases is further exacerbated by the widespread use of

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immunosuppressants, broad-spectrum antibiotics, and corticosteroids [3]. Among human fungal pathogens, *Candida* species (particularly *Candida albicans* and emerging non-*albicans* species) are the leading cause of invasive candidiasis [4], while *Aspergillus fumigatus* is the principal agent of invasive aspergillosis [5]. Other fungi, including *Histoplasma* spp. and *Pneumocystis jirovecii*, act as opportunistic pathogens in immunocompromised hosts, whereas *Cryptococcus neoformans* is a major cause of cryptococcosis, contributing significantly to morbidity and mortality, particularly among people living with HIV/AIDS [6, 7].

Similar to the pulmonary transmission of these fungal pathogens, *M. tuberculosis* predominantly affects the lungs, with infection typically occurring via inhalation of airborne droplet nuclei expelled by infected individuals. Similarly, some medically important fungi, such as *Cryptococcus neoformans*, are typically acquired through the inhalation of desiccated yeast cells, and *Histoplasma* species are acquired via inhalation of microconidia, with clinical disease developing particularly in immunocompromised hosts [8, 9].

In Nigeria, due to the absence of a centralized national body dedicated to monitoring fungal diseases, their actual prevalence remains unclear, and clinician awareness and clinical suspicion of fungal infections remain low [10].

Respiratory tract fungal infections caused by species such as *Histoplasma*, *Cryptococcus*, *Aspergillus*, and *Candida* have become a growing concern in recent years due to their increasing incidence, particularly among immunocompromised individuals [7, 11–13]. These fungal infections often present with clinical and radiological signs that closely mimic tuberculosis, complicating accurate diagnosis without proper laboratory testing. These overlapping clinical manifestations include chronic cough, weight loss, fever, and radiographic lung abnormalities. As a result, patients presenting with TB-like symptoms but actually infected with fungi are frequently misdiagnosed with TB and subjected to prolonged anti-TB therapy. This can lead to unnecessary drug exposure, an increased risk of drug-induced toxicity (especially hepatotoxicity), delayed treatment of the true underlying infection, and broader public health challenges [14, 15].

The assumption that such symptoms are solely attributable to *M. tuberculosis* infection may explain why fungal infections remain under-diagnosed in many TB-endemic regions, including Nigeria [6, 8].

Tuberculosis in Nigeria remains a public health challenge, with the country ranking among those with the highest burden of the disease worldwide [16]. However, it is highly likely that a notable proportion of patients with presumptive pulmonary tuberculosis may actually be harboring fungal pathogens, particularly pulmonary mycoses. The risk of misdiagnosis is further increased in settings where adequate diagnostic resources are limited and routine fungal screening is absent from TB diagnostic protocols.

Reportedly, the misattribution of fungal symptoms to TB or failure to consider fungal etiologies undermines the efforts of national TB programs in reducing TB morbidity and mortality [17]. Despite evidence demonstrating poor treatment responses, current clinical management guidelines frequently fail to address this microbiological complexity by routinely excluding antifungal coverage [14].

While histoplasmosis has been documented in presumptive TB patients in Calabar [6], there is no previous documented evidence specifically implicating *Cryptococcus* species in TB-like presentations in the study population. This gap in diagnostic and epidemiological data could undermine effective clinical management and disease surveillance; therefore, this study was undertaken to isolate and identify *Cryptococcus* species from patients with presumptive tuberculosis.

MATERIAL AND METHODS

Study area. Saint Benedict Tuberculosis, Leprosy and Rehabilitation Hospital is located in Moniaya-Ogoja, Cross River State, Nigeria. Ogoja is a Local Government Area situated in the northeastern part of the state, positioned close to the A4 highway. Geographically, it lies between latitudes 6°20′N and 6°40′N and longitudes 8°30′E and 9°00′E. The Local Government Area (LGA) covers an area of approximately 972 km², with an estimated 2018 population of 221,408, extrapolated using a population growth rate of 2.4% [18]. The area falls within the derived savanna ecological zone and experiences a humid climate marked by clearly defined wet and dry seasons, with an average annual rainfall of 1,500–2,500 mm [19]. The region is primarily rural, with agriculture being the major local economic activity [19]. Spatial mapping of the study location was performed using ArcGIS Pro (Esri, Redlands, CA, USA) as illustrated in Figure 1.

Study population. The study population consisted of patients referred from primary health centers across Cross River State who presented with a presumptive diagnosis of pulmonary tuberculosis.

Inclusion criteria. Patients of all ages and genders presenting with clinical features consistent with presumptive pulmonary tuberculosis at the time of enrollment.

Exclusion criteria. Patients with a prior confirmed diagnosis of tuberculosis, irrespective of current or prior anti-tuberculosis treatment status.

Study design. This was a cross-sectional, hospital-based investigation carried out at Saint Benedict Tuberculosis, Leprosy and Rehabilitation Hospital, Moniaya-Ogoja, Cross River State, Nigeria.

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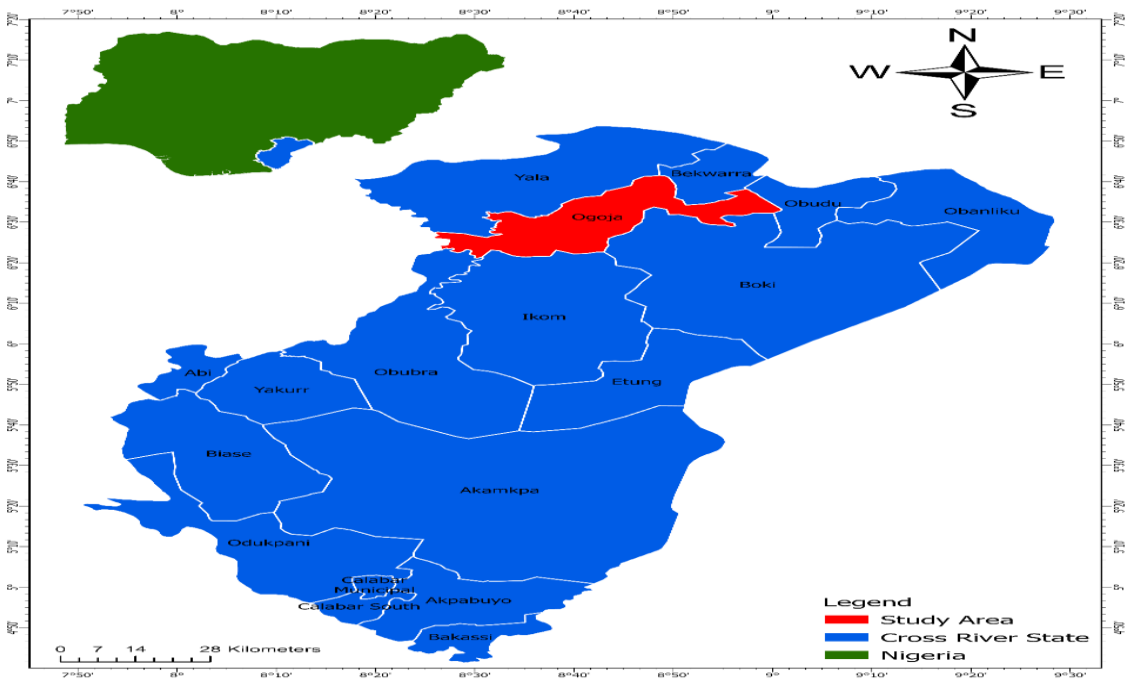

Fig. 1. Map of Cross River State, Nigeria, showing the location of the study area (Ogoja LGA)

Ethical consideration. The study received ethical approval from the Research and Ethics Committee of the Federal University Wukari (FUW/MCB/001-16/09-24) and Saint Benedict Tuberculosis, Leprosy and Rehabilitation Hospital, Moniaya-Ogoja (CDO/30-09/24). To ensure informed participation, patients were thoroughly briefed on the study's purpose, in accordance with the Declaration of Helsinki. Due to the low literacy rates prevalent in the study community and local traditions favoring oral agreements, a witness-attested verbal consent procedure was used in lieu of written consent, as approved by the ethics committee. Consequently, only patients who provided witnessed verbal consent were included in the study. Verbal consent was documented in a consent log, witnessed by an independent translator who facilitated interpretation to ensure participants fully understood the study information before agreeing to participate.

Determination of sample size. The sample size for the study population was determined using a standard population sample size formula [20].

$$N = \frac{Z^2 P(1-P)}{d^2} = 170.37$$

Where:

N = sample size

Z = critical z-value value at 95% confidence level (1.96)

P = anticipated prevalence of pulmonary fungal infections among presumptive TB patients in a similar Nigerian setting (0.127) [6]

D = margin of error (0.05)

In the absence of prior local data specifically on *Cryptococcus* prevalence among presumptive TB patients, a conservative estimated prevalence of 12.7% was used for sample size calculation, based on the reported overall burden of pulmonary fungal infections (including *Histoplasma* and other endemic mycoses) in similar Nigerian populations [6].

The calculated sample size of 170.37 was rounded up to 171; an additional 3 participants were included to account for potential sample exclusions, yielding a final recruitment target of 174 subjects. Although the study was conducted in a hospital setting with a finite population of presumptive tuberculosis patients, the calculated sample size represented < 5% of the estimated source population; therefore, the finite population correction factor was not applied, as it would have minimal effect on the required sample size.

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Media preparation. Sabouraud Dextrose Agar™ (Titan Biotech Ltd, Lot No.: MOF4FQ01) was used for fungal culture. The medium was prepared following the manufacturer's instructions: 62 g of powder was dissolved in 1 L of distilled water, mixed by swirling, then sterilized by autoclaving at 121°C for 15 minutes. After cooling to 47°C to preserve antibiotic activity, the medium was aseptically supplemented with chloramphenicol at 50 mg/L to suppress bacterial growth, then poured into sterile petri dishes and allowed to solidify.

Sample collection. A consecutive sampling approach was used, enrolling all eligible patients meeting inclusion criteria during the study period until the target sample size was reached. Morning sputum samples were collected in accordance with World Health Organization guidelines [21]. Patients were briefed on proper sputum collection technique, with emphasis on obtaining deep-lung secretions rather than saliva. They were instructed to sit upright or stand to facilitate deep breathing and productive coughing, and to take several deep breaths, inhaling fully and exhaling slowly, to help loosen respiratory secretions. After producing sputum, patients were instructed to expectorate directly into a sterile, wide-mouth container, ensuring the sample consisted of thick, mucoid sputum (not saliva) with a volume of 5–10 mL. Each container was tightly sealed to prevent leakage, individually labeled with a unique identification number, date, and collection time, immediately placed in a biohazard bag, and transported to the laboratory on ice packs. Samples were analyzed within two hours of collection; if delayed, samples were refrigerated at 4°C and processed within 24 hours. Laboratory analysis for *M. tuberculosis* was performed in a BSL-3-certified facility. Fungal culture and identification procedures for *Cryptococcus* species were conducted under BSL-2 conditions using a Class II biological safety cabinet, with appropriate personal protective equipment, including fluid-resistant gowns, N95 respirators, eye protection, and gloves.

Detection of *M. tuberculosis*. The microbiological examination of sputum samples for acid-fast bacilli was performed using the Ziehl-Neelsen (ZN) staining technique and the GeneXpert MTB/RIF assay, following the National Tuberculosis, Leprosy and Buruli Ulcer Control Program recommendations, consistent with the World Health Organization (WHO) guidance [21].

Ziehl-Neelsen staining. ZN staining is a conventional acid-fast technique used to detect *M. tuberculosis* complex and other acid-fast bacilli. A sterile disposable wooden applicator stick was used to prepare a direct smear by spreading a portion of sputum on a clean, dry, grease-free glass slide. The smear was air-dried, heat-fixed, and covered with carbolfuchsin stain. Using the hot ZN method, the slide was gently heated over a flame until steam rose, then left to stand for 5 minutes. It was then rinsed with gently flowing tap water and decolorized using 25% (*v/v*) sulfuric acid for two minutes, followed by another rinse with tap water. Methylene blue counterstain was applied and left for an additional two minutes. After a final gentle rinse under running water, the slide was air-dried and examined under oil immersion (×100) using a binocular microscope (Motic China Group Co., Ltd., European Division, 18/28 series) for the presence of acid-fast bacilli [22]. Each staining batch included appropriate positive (*M. tuberculosis* H37Rv) and negative (sterile saline) control smears to verify stain performance and reagent integrity. Slides were read independently by two trained microscopists, with each blinded to the other's interpretation, to minimize bias. Discrepant readings were resolved through repeat staining and joint re-examination. Inter-reader agreement for ZN smear interpretation was assessed; however, as no samples were positive for acid-fast bacilli, kappa statistics were not calculated for the final diagnostic outcome.

GeneXpert MTB/RIF assay. Briefly, 3 mL of the sample was combined with 6 mL of sample reagent (buffer) and mixed by vortexing. The mixture was then incubated at ambient temperature for 15 minutes, with an additional vortexing step at the 10-minute mark. From this processed sample, 2 mL was loaded into a multi-chambered, plastic Xpert MTB/RIF cartridge using a Pasteur pipette provided with the kit. The cartridge was scanned and inserted into the GeneXpert system (Cepheid, Sunnyvale, California, USA). After a processing time of approximately 2 hours, the results were automatically analyzed and displayed on the system monitor. The built-in internal quality controls of the GeneXpert MTB/RIF assay system (Sample Processing Control and Probe Check Control) were monitored for every run, and only results from runs that passed all internal control checks were included in the final diagnostic interpretation.

Isolation of fungus. Sputum samples were cultured on freshly prepared SDA containing chloramphenicol to inhibit bacterial growth. The plates were incubated at 37°C under aerobic conditions with maintained humidity for up to 7 days and monitored daily for fungal growth. Distinct fungal colonies were isolated by subculturing onto sterile SDA to obtain pure cultures [23].

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India ink staining. A small drop of India ink was carefully placed onto a clean, grease-free glass microscope slide. A loopful of the fungal colony was transferred onto the slide and mixed gently with the India ink. A clean coverslip was then placed over the preparation, avoiding air bubbles, and the slide was gently blotted with filter paper to create a thin, even film. The preparation was examined under a microscope using the ×100 oil-immersion objective. In this negative staining technique, the background appears dark while fungal cells and their polysaccharide capsules remain unstained, with the capsule observed as a clear halo against the dark background [22]. Standard reference descriptions from Cheesbrough [22] were used to validate colony morphology and microscopic features for fungal identification.

A sample was considered positive for *Cryptococcus* species only when both colonies with characteristic morphology were observed on SDA and India ink microscopy demonstrated encapsulated budding yeast cells. Specifically, positivity required growth of mucoid, creamy-white colonies morphologically consistent with *Cryptococcus* species on SDA, combined with demonstration of encapsulated budding yeast cells by India ink microscopy. Only sputum specimens of acceptable quality (as defined by macroscopic assessment for mucoid character and absence of predominant saliva) and obtained from patients with compatible clinical features were included.

The prevalence of *Cryptococcus* species was calculated as follows:

Prevalence of *Cryptococcus* species (%) = (Number of samples positive for *Cryptococcus* species / Total number of samples analyzed) × 100

Data were initially recorded in a field notebook at the point of collection and subsequently entered into an electronic database (Microsoft Excel).

Statistical analysis. Data were analyzed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize categorical variables as frequencies and percentages. The assumptions of the chi-square test were assessed, including the requirement that expected cell counts be ≥ 5 in at least 80% of cells. For contingency tables violating this assumption, the Fisher–Freeman–Halton exact test was applied. Prevalence estimates were reported with 95% confidence intervals (CIs), calculated for proportions using the exact binomial (Clopper–Pearson) method. Associations between age group, gender, and *Cryptococcus* infection status were evaluated using two-tailed chi-square or Fisher–Freeman–Halton exact tests as appropriate, with $P < 0.05$ considered statistically significant. Analyses were performed on complete cases only.

RESULTS

Tuberculosis screening results. A total of 174 patients with presumptive pulmonary tuberculosis were enrolled. Participants were referred from multiple primary health centers across Cross River State, and all presented with symptoms consistent with this clinical diagnosis. The most commonly reported clinical features included persistent cough lasting ≥ 4 weeks, sputum production, and fever, either alone or in combination. These symptoms formed the basis for clinical suspicion and subsequent laboratory investigations.

Samples were collected continuously from October 2024 to January 2025. Participants' ages spanned from pediatric cohorts to older adults (aged ≥ 1 year to ≥ 60 years), with a male majority. Of the 180 individuals approached, four refused to participate. A total of 176 samples were collected; two were excluded because they consisted predominantly of saliva rather than deep-lung sputum, yielding 174 specimens for analysis. *M. tuberculosis* was not detected in any of the 174 sputum samples by either Ziehl-Neelsen staining or the GeneXpert MTB/RIF assay (0/174; 95% CI: 0.0%–2.1%), indicating a detection rate of zero within the precision limits of the Clopper–Pearson exact binomial method.

Isolation and prevalence of *Cryptococcus* species. A total of 21 *Cryptococcus* species isolates were recovered, resulting in an overall prevalence of 12.1% among patients with presumptive pulmonary tuberculosis. On Sabouraud Dextrose Agar, colonies appeared smooth, creamy, and mucoid. Microscopic examination with India ink staining demonstrated encapsulated, budding yeast cells, morphologically consistent with *Cryptococcus* species.

Demographic distribution of *Cryptococcus* infection. Females exhibited a significantly higher prevalence of *Cryptococcus* detection ($\chi^2 = 39.426$, df = 1, $p < 0.001$) (35.2%, 19/54) compared with males (1.7%, 2/120). The observed concentration of *Cryptococcus* among individuals aged 30–39 and 50–59 years suggests a possible bimodal age distribution. Individuals in these age groups are more likely to be occupationally active and may have increased environmental exposure to desiccated yeast. Additionally, advancing age is associated with immunological changes and a higher prevalence of underlying conditions that may predispose individuals to opportunistic fungal infections. However, the absence of immunological and occupational data in this study limits further interpretation of this pattern. Although no *Cryptococcus* species were detected among participants aged 40–49 years, infection rates varied across other age categories; however, the association between age and infection status was not statistically significant. Because 50% of cells in the age group × infection status contingency table had expected counts less than 5, the assumptions of the chi-square test were violated for this analysis; therefore, the Fisher–Freeman–Halton exact test was applied. No statistically significant association was observed ($P = 0.199$) (Table 1).

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Table 1. Distribution of *Cryptococcus* species detection by gender and age group among presumptive pulmonary tuberculosis patients (N = 174)

Variable	Category	Number examined	Number positive (%) [95% CI]	Test statistic	*P*-value
Gender	Male	120	2 (1.7%) [0.2–6.0]	$\chi^2 = 39.426$	< 0.001
	Female	54	19 (35.2%) [22.7–49.4]		
Age (years)	1–9	5	0 (0.0%) [0.0–52.2]		
	10–19	35	5 (14.3%) [4.8–30.3]		
	20–29	40	2 (5.0%) [0.6–16.9]		
	30–39	53	10 (18.9%) [9.6–32.0]	Fisher–Freeman–Halton 7.681	0.199
	40–49	15	0 (0.0%) [0.0–21.8]		
	50–59	20	4 (20.0%) [5.7–43.7]		
	≥ 60	6	0 (0.0%) [0.0–45.9]		
Total		174	21 (12.1%) [7.6–18.0]		

Note: Pearson's chi-square test was applied for gender analysis (2 × 2 contingency table; all expected cell counts ≥ 5). The Fisher–Freeman–Halton exact test was applied for age-group analysis because 50% of cells (7 of 14) in the 7 × 2 contingency table had expected counts < 5, violating the chi-square assumption. Ninety-five percent confidence intervals (CIs) for proportions were calculated using the exact binomial (Clopper–Pearson) method. *Cryptococcus* is italicized per taxonomic convention. CI = confidence interval; df = degrees of freedom.

DISCUSSION

This study enrolled 174 patients with presumptive pulmonary tuberculosis from multiple primary health centers across Cross River State, referred to Saint Benedict Tuberculosis, Leprosy and Rehabilitation Hospital. No *M. tuberculosis* was detected using Ziehl-Neelsen staining or the GeneXpert MTB/RIF assay, yielding a prevalence of 0% (95% CI: 0%–2.1%). *Cryptococcus* species were recovered from 21 sputum samples, resulting in an overall prevalence of 12.1% (95% CI: 7.6–18.0%). Colonies exhibited typical morphology on Sabouraud Dextrose Agar, and India ink staining demonstrated morphological features consistent with encapsulated yeast cells, supporting presumptive identification. Notably, females had a significantly higher prevalence (35.2%, 19/54; 95% CI: 22.7–49.4%) compared with males (1.7%, 2/120; 95% CI: 0.2–6.0%; $\chi^2 = 39.426$, $P < 0.001$). Age-specific analysis suggested a possible bimodal distribution with peaks in the 30–39 and 50–59 years age groups, while 0 of 15 participants (0.0%) in the 40–49 years age group tested positive; however, subgroup sample sizes limit definitive conclusions. Age was not significantly associated with infection status (Fisher–Freeman–Halton exact test = 7.681, $P = 0.199$).

The prevalence of *Cryptococcus* species observed in this study (12.1%) is comparable in magnitude to the prevalence of *Histoplasma* infection (12.7%) reported among presumptive pulmonary tuberculosis patients in a similar Nigerian population [6]. However, studies from Nigeria and other African settings report a substantially higher overall burden of pulmonary fungal infections among presumptive pulmonary tuberculosis patients. In Port Harcourt, Nigeria, Baadom *et al*. [24] reported an overall fungal prevalence of 64.3% (225/350) among presumptive TB patients, though *Cryptococcus* species constituted only a subset of these isolates. In southwestern Uganda, Njovu *et al*. [25] reported that 80 out of 113 participants (70.7%) with clinical features of pulmonary tuberculosis had evidence of pulmonary fungal pathogens. Similarly, in Ethiopia, Bitew and Bati [26] detected fungal growth in 75.9% of sputum samples, although *C. neoformans* constituted only 1.0% of isolates. The variation in prevalence and species distribution across studies likely reflects differences in environmental exposures, host immune status, healthcare-seeking behavior, antifungal or antibiotic use prior to sampling, and the diagnostic methods employed (culture-based methods versus antigen detection or molecular assays). Despite this heterogeneity, these findings consistently demonstrate that fungal pathogens are a frequent cause of respiratory illness among presumptive pulmonary tuberculosis patients and represent a significant source of diagnostic challenge in TB-endemic regions, emphasizing the importance of incorporating fungal diagnostics into routine TB evaluation algorithms.

The moderate prevalence of *Cryptococcus* species in this study emphasizes the significance of fungi as an often-overlooked cause of respiratory illness, especially among individuals exhibiting symptoms indicative of tuberculosis. This finding is clinically relevant, as it highlights the potential for misdiagnosis and the delayed or under-recognition of pulmonary cryptococcal infections in settings where TB is highly endemic and diagnostic resources are limited. Moreover, given the widespread incidence of TB in high-burden areas, the clinical presentation of pulmonary cryptococcal infection can be virtually indistinguishable from that of TB. This diagnostic challenge is further compounded in clinical settings where TB is often the primary focus and fungal diagnostics are not routinely available [6, 27]. This reinforces the importance of considering fungal infections as potential causes of persistent respiratory symptoms and performing routine mycological investigations in these patients, especially when TB test results are negative.

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The significant gender disparity observed in the prevalence of *Cryptococcus* detection in this study, with females exhibiting a higher positivity rate than males, is consistent with findings from Njovu *et al*. [25], who reported that females accounted for a higher proportion of pulmonary fungal pathogens among patients with presumptive pulmonary tuberculosis. This finding should be interpreted with caution, however, as key confounding variables such as HIV status, immune status, and environmental exposures were not assessed. Multiple factors may contribute to the increased susceptibility of females to *Cryptococcus* colonization or infection in this cohort. Given that *Cryptococcus* is a globally distributed opportunistic fungal pathogen primarily originating from environmental sources such as soil, decaying wood, and bird droppings [28, 29], the observed higher positivity among females (19/54; 35.2%) compared with males (2/120; 1.7%) is striking and warrants careful interpretation. One contributing factor may be the smaller sample size of females relative to males, which, while accounted for in the chi-square analysis, limits the precision of prevalence estimates for this subgroup. Future studies incorporating detailed occupational, environmental, and pregnancy status, alongside comprehensive immune profiling, are needed to clarify the factors underlying this gender pattern.

The observed concentration of *Cryptococcus* detection among individuals aged 30–39 and 50–59 years presents a bimodal age distribution. These age groups are more likely to be occupationally active and may have increased environmental exposure to desiccated yeast. Additionally, advancing age is associated with immunological changes and a higher prevalence of underlying conditions that may predispose individuals to opportunistic fungal infections [30]. However, the absence of immunological and occupational data in this study limits further interpretation of this pattern.

The absence of *M. tuberculosis* detection among patients with presumptive pulmonary tuberculosis in this study is unusual but may be attributed to several factors. The referral criteria could have contributed to the inclusion of patients with respiratory symptoms that mimic TB, such as bacterial pneumonia, asthma, chronic bronchitis, or post-viral cough syndromes. In many primary health care and peripheral facilities, persistent cough is often used as the main criterion for referral, even when other cardinal features of TB such as night sweats, weight loss, or hemoptysis are absent. In addition, prior empirical use of anti-tuberculosis therapy, common in many settings before referral, may have reduced mycobacterial load below detectable levels. Diagnostic limitations of the GeneXpert assay, particularly in cases of paucibacillary disease, early-stage infection, or extrapulmonary tuberculosis, may further account for the 0% prevalence of *M. tuberculosis*. As a result, a substantial proportion of patients with a low probability of TB might have been referred for testing. Prior exposure to anti-tuberculosis therapy cannot be completely excluded; this may also contribute to the observed 0% prevalence of *M. tuberculosis*. Alternative infectious etiologies, such as fungal pathogens, may also explain TB-like clinical presentations, as demonstrated by the isolation of *Cryptococcus* species. In this study, identification of *Cryptococcus* was presumptive, as it was based solely on culture characteristics and India ink staining, without confirmatory biochemical or molecular testing.

The cross-sectional design of this study limits the ability to assess temporal relationships, disease progression, or causal associations. Additionally, being conducted at a single referral center may restrict the generalizability of the findings to other populations or settings. Furthermore, clinical correlation was limited, as detailed data on participants' immune status, comorbidities, HIV status, and occupational or environmental exposures were not evaluated. Age-specific prevalence estimates were based on small subgroup sizes, which limits the reliability of percentage estimates within these categories. Given these small subgroup sizes, a single additional positive case would markedly alter the calculated prevalence; therefore, age-stratified findings should be interpreted with caution. The moderate prevalence of *Cryptococcus* species in sputum obtained in the present study suggests a possible association with lower respiratory tract disease in individuals without tuberculosis; however, isolation from sputum alone does not confirm active infection without clinical, radiological, or antigenic correlation.

The identification of *Cryptococcus* species as a high-priority pathogen by the World Health Organization underscores the clinical importance of the organism investigated in this study [1]. The inclusion of *C. neoformans* in the WHO Fungal Priority Pathogens List reflects its association with invasive fungal disease, increasing antifungal resistance, and significant management challenges, particularly among immunocompromised and medically vulnerable populations [1]. The expanding population at risk for invasive fungal disease, driven by immunosuppressive therapies, chronic comorbidities, and emerging risk groups provides important context for interpreting the findings of this study [31, 32]. The observed relevance of *Cryptococcus* species in this setting aligns with global concerns regarding limited diagnostic access, constrained treatment options, and rising antifungal resistance, especially in low- and middle-income countries [1, 33]. Resistance is driven in part by inappropriate antifungal use across the One Health spectrum, which considers the interconnected health of humans, animals, and the environment [34]. Given that current clinical management relies on only four major systemic antifungal classes, all associated with toxicity, drug interactions, and prolonged treatment courses [35–37], the findings of this study reinforce the need for early detection and informed clinical management of *Cryptococcus* infection to improve patient outcomes [33, 35].

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All 21 *Cryptococcus*-positive patients were referred for further clinical evaluation and management. However, this study did not systematically collect follow-up data on treatment outcomes.

In conclusion, the findings of this study emphasize the need for integrated diagnostic approaches that encompass fungal screening in patients with presumptive pulmonary tuberculosis, particularly in situations where fungal infections are often neglected. Future studies should address key gaps by incorporating molecular epidemiological analyses, evaluating associations with HIV and other immunosuppressive conditions, adopting prospective study designs, and performing antifungal susceptibility testing to guide effective clinical management. Furthermore, resistance surveillance and post-referral monitoring should be incorporated to better understand the clinical course and impact of *Cryptococcus* infection. Improved awareness among healthcare providers and enhanced laboratory capacity for fungal diagnosis are critical; this can be achieved through targeted training programs, routine continuing medical education, incorporation of fungal diagnostics into standard clinical protocols, and investment in affordable, rapid diagnostic tools to reduce misdiagnosis and ensure appropriate treatment regimens.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

AI DISCLOSURE

AI-assisted language editing tools were used solely for grammar, spelling, and clarity improvements. All scientific content, analysis, and interpretations are the sole responsibility of the authors. The authors retain full responsibility for the scientific content and integrity of this manuscript.

DATA AVAILABILITY

The datasets generated and analyzed during this study, including patients' demographic information and laboratory diagnostic results, are available from the corresponding author upon reasonable request. Access to the data is subject to ethical approval and institutional restrictions to protect participant confidentiality.

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AUTHORS' CONTRIBUTIONS

CNA: conceptualization, project administration, supervision, writing – review & editing, methodology, investigation; EG: data curation, methodology, investigation, formal analysis, visualization, writing – original draft; STB: data curation.

ETHICS STATEMENT

Ethical approval for this study was obtained from the Research and Ethics Committee of the Federal University Wukari (Approval No. FUW/MCB/001-16/09-24) and Saint Benedict Tuberculosis, Leprosy and Rehabilitation Hospital, Moniaya-Ogoja (Approval No. CDO/30-09-24).

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