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Short Communication **Open Access**

Prevalence of pulmonary mycoses among patients with suspected pulmonary tuberculosis at the Infectious Disease Hospital, Kano, Nigeria

*Salim, A. B., and Nabila, M. I.

Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, PMB 3011, Kano, Nigeria
*Correspondence to: ahmadbsalim@gmail.com; +2348033731917

Abstract:

Background: Opportunistic fungal infections have been implicated among patients with tuberculosis (TB). Coexistence of pulmonary mycoses and pulmonary TB is of public health concern as they exhibit similar clinical manifestations among infected patients, which contributes to increased misdiagnosis and makes management difficult, especially in TB-prone regions like Kano, Nigeria. Thus, this study was aimed at determining the prevalence of pulmonary mycoses among patients with suspected pulmonary TB attending the Infectious Disease Hospital (IDH), Kano State, Nigeria.
Methodology: Sputum samples were collected from a total of 155 patients with clinically suspected TB. The samples were analysed for the detection of *Mycobacterium tuberculosis* (MTB) using GeneXpert in line with the National Tuberculosis and Leprosy Control Program (NTBLCP) standard operating procedures and for the isolation and identification of pulmonary mycoses using cultural methods on Sabouraud Dextrose Agar (SDA) and microscopy techniques including lactophenol cotton blue stain, Gram stain, 10% KOH wet mount and germ tube tests.
Results: Of the total of 155 study participants recruited into the study, 67 (43.2%) were males and 88 (56.8%) were females. Of these, 88 (56.8%) and 31 (20.0%) were positive for pulmonary mycoses by culture and PTB by GeneXpert assay, respectively, while 9.0% (n=16) had pulmonary mycoses-PTB co-infection**.** *Aspergillus* spp was the most frequent fungus (n=76, 86.4%) identified while *Cryptococcus neoformans* and *Histoplasma capsulatum* were the least frequent, each with 2.3% (n=2).
Conclusion: The high rate of fungal infection (56.8%) among patients with suspected pulmonary TB is a significant public health challenge. There is need for routine screening of all patients with suspected TB for pulmonary mycoses, especially in high TB-burden regions, as this will contribute to improved management and reduction in morbidity.

Keywords: Pulmonary mycoses, pulmonary tuberculosis, GeneXpert, fungal culture, co-infection

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Prévalence des mycoses pulmonaires chez les patients présentant une suspicion de tuberculose pulmonaire à l'hôpital des maladies infectieuses de Kano, au Nigéria

*Salim, A. B., et Nabila, M. I.

Département de microbiologie, Faculté des sciences de la vie, Université Bayero, Kano, PMB 3011, Kano, Nigéria
*Correspondance à: ahmadbsalim@gmail.com; +2348033731917

Résumé:

Contexte: Des infections fongiques opportunistes ont été observées chez les patients atteints de tuberculose (TB).

La coexistence de mycoses pulmonaires et de tuberculose pulmonaire constitue un problème de santé publique, car ces deux affections présentent des manifestations cliniques similaires chez les patients infectés, ce qui contribue à l'augmentation des erreurs de diagnostic et complique la prise en charge, notamment dans les régions à forte prévalence de tuberculose comme Kano, au Nigéria. Cette étude visait donc à déterminer la prévalence des mycoses pulmonaires chez les patients présentant une suspicion de tuberculose pulmonaire et consultant à l'hôpital des maladies infectieuses (IDH) de l'État de Kano, au Nigéria.

Méthodologie: Des échantillons d'expectorations ont été prélevés chez 155 patients présentant une suspicion clinique de tuberculose. Ces échantillons ont été analysés pour la détection de *Mycobacterium tuberculosis* (MTB) par la technique GeneXpert, conformément aux procédures opératoires standard du Programme national de lutte contre la tuberculose et la lèpre (NTBLCP), et pour l'isolement et l'identification des mycoses pulmonaires par culture sur gélose Sabouraud dextrose (SDA) et par microscopie (coloration au bleu coton lactophénol, coloration de Gram, examen microscopique direct après coloration au KOH à 10% et test de germination).

Résultats: Sur les 155 participants inclus dans l'étude, 67 (43,2%) étaient des hommes et 88 (56,8%) des femmes. Parmi ces patients, 88 (56,8%) et 31 (20,0%) étaient positifs respectivement pour les mycoses pulmonaires (culture) et la tuberculose pulmonaire (test GeneXpert), tandis que 9,0% (n=16) présentaient une co-infection mycoses pulmonaires-tuberculose pulmonaire. *Aspergillus* spp était le champignon le plus fréquemment identifié (n=76, 86,4%), tandis que *Cryptococcus neoformans* et *Histoplasma capsulatum* étaient les moins fréquents, chacun représentant 2,3% des cas (n=2).

Conclusion: La forte prévalence d'infections fongiques (56,8%) chez les patients présentant une suspicion de tuberculose pulmonaire constitue un enjeu majeur de santé publique. Un dépistage systématique des mycoses pulmonaires chez tous les patients suspects de tuberculose est nécessaire, en particulier dans les régions à forte prévalence de tuberculose, afin d'améliorer la prise en charge et de réduire la morbidité.

Mots-clés: Mycoses pulmonaires, tuberculose pulmonaire, GeneXpert, culture fongique, co-infection

Introduction:

Pulmonary mycoses are fungal infections of the lungs that resemble pulmonary tuberculosis (PTB). Opportunistic fungal infections have been implicated in the rise of pulmonary mycoses among patients with TB, which poses a diagnostic challenge. Many other factors are also implicated in the increase of these fungal infections, but the most common causes remain PTB, HIV/AIDS, and extensive use of immunosuppressive drugs (1). Extensive use of antibiotics and steroids has also resulted in increasing trends of these fungal infections globally.

In the environment, there is a significant number of fungi and their spores which colonize the lung parenchyma by various modes such as inhalation, blood route, or by reactivation of a dormant infection and as such pose a major challenge in patients with PTB (2). Common fungal pathogens associated with pulmonary mycoses isolated from patients with PTB, include *Aspergillus* spp such as *Aspergillus niger* and *Aspergillus fumigatus*, *Histoplasma capsulatum* and *Cryptococcus neoformans* (3).

Tuberculosis is a potentially serious chronic lung disease that is caused by *Mycobacterium tuberculosis* and characterized by injury to the lung parenchyma with a granulomatous lesion along with epithelioid macrophages and Langhans giant cells with fibroblasts and collagenous tissue, as well as hallmark caseous necrosis in the centre (4). There have been studies that suggest that the chronic nature of tuberculosis tends to be more lethal when it is associated with annexation by parasitic fungi, as not only does it make the TB more virulent, but it also adds to various complications and even death (5).

Coexistence of pulmonary mycoses and PTB contribute to increased misdiagnosis, mismanagement and mistreatment by physicians because they do not show specific clinical manifestations, and as such leads to high morbidity and considerable mortality (6). Identification and characterization of fungal pulmonary pathogens among TB suspects would be of immense importance because it will lead to improved proper diagnosis where pulmonary mycoses are diagnosed and/or excluded. Thus, proper medications and managements can be adjusted accordingly, enhancing adherence and good treatment outcomes, especially in TB-prone regions such as Kano, Nigeria.

This study therefore was aimed at determining the prevalence of pulmonary mycoses among patients with suspected PTB attending the Infectious Disease Hospital (IDH), Kano, Nigeria.

Materials and method:

Study area:

The study was conducted at the Infectious Diseases Hospital (IDH), Kano, Nigeria. Located along France road within Kano metropolis, IDH is the main hospital that is designated by Kano State Government for treatment of infectious diseases such as TB, HIV/AIDS malaria, meningitis etc.

Kano city is located between longitude 8.500°E and latitude 11.500°N. It consists pri-

marily of Sudan savanna type vegetation, with mean annual rainfall of 800-900 mm, and temperature that ranges between 25–40°C. The hospital receives patients from the whole of northern parts of Nigeria, and serves as referral centre for TB and HIV/AIDS control activities and other infectious diseases, not only for Kano and other neighbouring States but also for Niger and Cameroon Republics.

Study design and period:

This was a cross sectional, hospital-based study of 155 patients with suspected pulmonary TB aged 10 years and above in January and February 2024, to determine the prevalence of pulmonary mycoses and to detect *M. tuberculosis* (by GeneXpert) among them.

Ethical approval, patient consent and questionnaire:

Ethical approval was obtained from the Health Research Ethics Committee of Kano State Ministry of Health (NHREC/17/03/2018). Prior to specimen collection, informed consent form was designed and signed by all recruited patients. A structured questionnaire capturing socio-demographic data (age, sex, etc) and risk factors for pulmonary mycoses (cigarette smoking, HIV etc) was administered to all consented participants.

Sample size determination:

The Fischer formula, $N=Z^2pq/d^2$ was used to determine the sample size of participants, where N=sample size, Z=standard normal distribution at 95% confidence interval (=1.96), p=prevalence of 9.7% (0.097) from the study of Ocansey et al., (7), q=1–p, and d=allowable error of 5% (0.05). This gave a calculated sample size of 134.6, which was adjusted to 155.

Inclusion and exclusion Criteria:

This included both male and female participants 10 years and above who presented with clinical symptoms and signs of PTB (chronic cough, night sweats, weight loss, fever, and history of contact with person with chronic cough etc) irrespective of HIV status, and consented to participate in the study. Patients with chronic conditions such as diabetes mellitus, chronic obstructive pulmonary diseases, and patients with a history of smoking or alcohol use, were also included.

Patients with extrapulmonary complications or miliary TB, those on antifungal drugs and those who did not consent to participate in the study, were excluded.

Sample collection:

About 4 ml of early morning sputum sample was collected from each study participant into a sterile, wide-neck, transparent plastic leak-proof container in line with NTBLCP standard operating procedures (8), and transported to the medical microbiology unit of the laboratory department of IDH for processing on the same day of collection. Samples not processed on the same day were stored in the refrigerator at 4–8°C until processed.

Detection of *M. tuberculosis* in sputum sample by GeneXpert assay:

Mycobacterium tuberculosis from the sputum samples was detected using the GeneXpert assay machine (Cepheid, Sunnyvale, CA, USA) according to the manufacturer's instructions. Briefly, the sputum sample was mixed with sample reagent in a ratio of 2:1 and allowed to settle at room temperature. Subsequently, 2 ml of the mixture was transferred into the GeneXpert cartridge and loaded into the GeneXpert machine for 2 hours. The results were read and recorded as MTB not detected, MTB detected, RIF resistance not detected, or RIF resistance detected (9).

Isolation and identification of pulmonary fungal pathogens (PFP):

All specimens were analysed by direct microscopy using KOH 10% mount, Gram stain and culture for isolation and detection of fungi. Fungal identification was done by microscopic examination of lactophenol cotton blue (LPCB) stain slides and by germ tube test.

Potassium hydroxide (KOH) 10% wet mounts:

With the aid of Pasteur pipette, a large drop of KOH 10% was placed on the centre of a clean glass slide. A small portion of the purulent part of sputum sample was transferred into the KOH drop with a sterile wire loop and was mixed well, covered with cover slip and placed in a moist chamber at room temperature for 30 minutes. The preparation was then examined under a compound light microscope using low power (10x and 40x objectives) for the presence of fungal elements (10).

Gram stain for yeast identification:

Briefly, a smear was made from the sputum samples on a clean glass slide. The cells and cell fragments were dried on the slide and stained with standard Gram stain as described in Cheesbrough (10). The presence of pseudo hyphae in the sputum was seen as Gram-positive yeast-like cells.

For differential staining, the slide was treated for 5 min with 1% phosphomolybdic acid, washed, and stained for 0.5 min with 1% aqueous methyl green. Whole or intact yeast cells appeared dark purple or black, while broken yeast cells with exposed cytoplasm were stained light purple.

Fungal culture:

Sputum samples were streaked onto Sabouraud Dextrose Agar (SDA) supplemented with and without antibiotic (gentamicin 40 mg/L) to isolate pure culture. The plates were inverted and incubated at 25–30 °C. The fungal growth on SDA plates was examined after 7 days and held for 4 weeks before being reported as negative. The morphology, texture, growth rate, colony/cottony surface, and pigmentation on the surface of SDA tubes were recorded. The fungal isolates on culture were identified using standard mycological procedures. A routine examination of the culture plates was done to exclude any fungal growth at least 3 times a week (10).

Lactophenol cotton blue (LPCB) stain:

A section of small fragment of fungal growth was transferred on to a drop of ethanol placed on a clean, grease-free glass slides. Few drops of LPCB were then added and the slide covered with cover slip. Excess LPCB stain was removed with blotting paper. The preparation was then placed in a moist chamber and kept at room temperature for 30 minutes. The slide was viewed under the microscope using 10x and 40x objectives. The features and characteristics of fungal pathogens seen under the microscope were compared with established characteristic fungal features using mycology atlases.

Germ tube test:

Candida albicans was identified by the germ tube test. Briefly, yeast colonies from the SDA plates were suspended in human serum in a test tube and incubated at 37°C for 3 hours. After observing a faintly turbid suspension, the suspension was examined under the light microscope using 10x and 40x objectives to detect the presence or absence of germ tubes. The result was positive for *C. albicans* if germ tubes arose from the yeast cell and showed matching walls without any narrowing at their point of origin (10).

Statistical analysis of data:

The OpenEPI (version 3.01) statistical online tool was used for data analysis. Chi square test and Odds ratio at 95% confidence interval were used to determine association between categorical variables. A p value < 0.05 was considered statistically significant.

Results:

Prevalence of pulmonary mycoses in the study population:

Table 1 shows the prevalence of pulmonary mycoses among the study population with respect to socio-demographic characteristics (sex and age group). Of the total 155 study participants, 67 (43.2%) were males and 88 (56.8%) were females. A total of 88 (56.8%) participants' sputum samples were positive for pulmonary fungal pathogen (PFP). Females had a higher PFP prevalence of 59.1% (n=52/88) compared to males with 53.7% (n=36/67), but the PFP prevalence difference was not statistically significant (x^2=0.4452, p=0.5046).

Participants in the age groups 20-29 years and 50-59 years had higher PFP prevalence of 67.6% (n=23/34) and 63.0% (n=17/27) respectively, while age groups 10-19 years and ≥60 years had lower PFP prevalence of 50.0% (n=15/30) and 46.7% (n=7/15), respectively. However, the difference in PFP prevalence with respect to age was not statistically significant (x^2=3.534, p=0.6183).

Frequency distribution of fungi isolates among population with pulmonary mycoses:

Among the participants with pulmonary mycoses, *Aspergillus* spp accounted for the highest frequency (n=76, 86.4%) while *C. neo formans* and *H. capsulatum* had the lowest frequencies, each with 2.3% (n=2). The frequency of *Aspergillus* spp was higher in females (n=47, 90.4%) compared to males (n=29, 80.6%) but the difference was not statistically significant (OR=0.4407, 95% CI=0.1278-1.520, p=0.2176). However, higher frequency of *C. albicans* was recorded among male (n=5, 13.9%) than female (n=3, 5.8%) participants, although the difference was also not statistically significant (OR=2.634, 95% CI=0.5874-11.815, p=0.2637).

Prevalence of co-infection of pulmonary myco ses and PTB in the study population:

Of the sputum samples of 155 participants analyzed, 88 (56.8%) and 31 (20.0%) were positive for pulmonary mycoses (by culture) and PTB (by GeneXpert) respectively. In all, 14 (9.0%) were positive for both pulmonary mycoses and PTB, of which males accounted for 6 (8.9%) and females 8 (9.1%).

Table 1: Prevalence of pulmonary mycoses among patients with presumptive pulmonary tuberculosis with respect to sociodemographic characteristics

Characteristics	Fungi culture		Total	x^2	*p* value
	No positive (%)	No Negative (%)			
Sex					
Male	36 (53.7)	31 (46.3)	67	0.4452	0.5046
Female	52 (59.1)	36 (40.9)	88		
Age group (years)					
10-19	15 (50.0)	15 (50.0)	30	3.534	0.6183
20-29	23 (67.6)	11 (32.4)	34		
30-39	14 (53.8)	12 (46.2)	26		
40-49	12 (52.2)	11 (47.8)	23		
50-59	17 (63.0)	10 (37.0)	27		
>60	7 (46.7)	8 (53.3)	15		
Total	**88 (56.8)**	**67 (43.2)**	**155**		

x^2 = Chi square

Table 2: Frequency distribution of fungi isolates among patients with pulmonary mycoses in the study population

Fungi isolate	Number of patients		Total (%)	OR (95% CI)	P value
	Male	Female			
Aspergillus spp	29 (80.6)	47 (90.4)	76 (86.4)	0.4407 (0.1278 - 1.520)	0.2176
Candida albicans	5 (13.9)	3 (5.8)	8 (9.1)	2.634 (0.5874 - 11.815)	0.2637
Cryptococcus neoformans	1 (2.8)	1 (1.9)	2 (2.3)	1.457 (0.08811 - 24.099	1.0000
Histoplasma capsulatum	1 (2.8)	1 (1.9)	2 (2.3)	1.457 (0.08811 – 24.099)	1.000
Total	**36 (40.9)**	**52 (50.1)**	**88 (100.0)**		

OR = Odd ratio; CI=Confidence interval

Table 3: Prevalence of pulmonary mycoses and pulmonary tuberculosis co-infection among the study population

Gender	No of pulmonary mycoses by culture (%)	No of pulmonary TB by GeneXpert (%)	No of pulmonary mycoses-pulmonary TB co-infection (%)
Male (n=67)	36 (53.7)	16 (23.9)	6 (8.9)
Female (n=88)	52 (59.1)	15 (28.8)	8 (9.1)
Total (n=155)	**88 (56.8)**	**31 (20.0)**	**14 (9.0)**

Discussion:

The respiratory tract is the most common site for developing fungal infection and in most cases, colonization is the first step in the progression to pulmonary fungal infection. In this study, sputum samples collected from a total of 155 patients with suspected pulmonary TB were examined, in which 56.8% were positive for pulmonary mycoses, which is similar but slightly lower than the 73.6% reported by Sani et al., (11) among presumptive TB cases in Gombe, Nigeria. Similarly, an Egyptian study also showed that the prevalence of fungal pneumonia in respiratory intensive care unit was 66.7% (12). The slightly lower prevalence rate obtained, might be due to the smaller sample size of this study (samples were collected for a period of 1 month only) when compared to the other two. In all cases, the prevalence of pulmonary mycoses was high.

The male-to-female ratio of 36/52 among the 88 patients with pulmonary mycoses in this study agrees with 60/90 male-to-female ratio reported by Jahromi and Khaksar (13). Sex-related distribution of fungal infections, according to Hidalgo and Vazquez (14), as cited by Ndukwu et al., (15) are equal. Other studies such as that of Yahaya et al., (16) and

Bansod and Rai (3), have reported higher prevalence among males than females, attributing that males are more vulnerable to fungal infections because they are more exposed to external environment where fungi and their spores are in significant numbers. It could also be due to the higher number of female patients in the overall study population, since females generally have higher hospital attendance than males.

Fungal infections of the respiratory tract by large are considered to be identical with invasive pulmonary infections caused by *Aspergillus* spp (2). Our findings are in consistent with this report because out of 155 mycelial fungi recovered in the present study, *Aspergillus* spp accounted for the highest (n=76, 86.4%) proportion while *C. neoformans* and *H. capsulatum* had the lowest, each with 2.3%. Among species of *Aspergillus*, *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus* are pathogenic species to man. Most previous studies reported that *A. fumigatus* is the most common cause of pulmonary mycosis (11,17,18).

Candida albicans was the second-most common fungal pathogen isolated in this study, accounting for 9.1%. This is similar to the study of Sani et al., (11) in which *C. albicans* was also reported to be the second-most prevalent fungal isolate and common in co-infection with pulmonary TB. *Candida albicans* is an opportunistic fungal pathogen that affects individuals with a prior history of pathogenic infectious disease with prolonged treatment therapy (19). The high occurrence of this species among pulmonary TB patients indicates fungal colonization due to the extended use of antibiotics. *Candida albicans* was previously reported and has remained the most frequently isolated *Candida* species in the clinical setting (20).

While the prevalence of 56.8% of pulmonary mycosis in presumptive PTB patients in this study is quite similar to the prevalence of 50.0% for chronic pulmonary aspergillosis in patients with presumptive pulmonary TB in Ghana (7), the prevalence of pulmonary mycoses in confirmed pulmonary TB patients is considerably lower. For example, the prevalence of pulmonary fungal pathogen in patients with confirmed pulmonary TB at the Federal Teaching Hospital, Gombe, Nigeria was 6.0% (11), similar to 9.0% that was obtained in our current study. The similarity in the prevalence of co-infection might be due to the study area and study population, where both locations are in northern part of Nigeria having similar climates and environmental conditions with population of same or similar cultural practices. As emphasized by Sule et al., (21), the prevalence of pulmonary mycoses among patients with confirmed pulmonary TB (co-infection) progressively increases, majorly, as a result of anti-TB drugs with non-specific action and depressed immunity, which enable fungi to proliferate.

Conclusion:

From the findings of this study, TB patients are in many cases co-infected with opportunistic pulmonary mycotic agents with *Aspergillus* spp being the most frequent fungal pathogen but also by *C. albicans, C. neoformans* and *H. capsulatum.* There is need for routine screening of all patients with suspected pulmonary TB for pulmonary mycoses, especially in high TB-burden regions, as this will contribute to improved management, and reduction in mortality and morbidity.

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Contributions of authors:

Both authors contributed to conceptualization and design of the study, data analysis and interpretation of results. NMI performed laboratory analysis and ABS drafted the manuscript.

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Conflict of interest:

Authors declare no conflict of interest.

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