Open Forum Infectious Diseases

MAJOR ARTICLE

Prevalence of Histoplasmosis and Molecular Characterization of *Histoplasma* species in Patients with Presumptive Pulmonary Tuberculosis in Calabar, Nigeria

Bassey E. Ekeng,[1,2] Rita O. Oladele,[1,3] Ubleni E. Emanghe,[2] Ernest A. Ochang,[2] and Tatfeng Y. Mirabeau[4]

[1]Medical Mycology Society of Nigeria, Lagos, Nigeria, [2]Department of Medical Microbiology and Parasitology, University of Calabar Teaching Hospital, Calabar, Nigeria, [3]Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria, and [4]Department of Medical Laboratory Science, College of Health Sciences, Niger Delta University, Amassoma, Nigeria

Background. Several case reports abound in literature about cases of histoplasmosis misdiagnosed as tuberculosis (TB). Nigeria is one of the highest TB-burdened countries, but data on histoplasmosis in Nigeria are sparse in the literature. The aim of this research was to investigate patients with presumptive pulmonary TB in Calabar, Nigeria, for histoplasmosis.

Methods. This was a descriptive cross-sectional study of 213 participants with presumptive diagnosis of pulmonary TB between April 2020 and March 2021. Urine samples were collected from selected patients for *Histoplasma* antigen test using enzyme immunoassay kits, while sputum samples were collected for GeneXpert test for confirmed diagnosis of TB and conventional polymerase chain reaction (PCR) for the diagnosis of histoplasmosis.

Results. Of the 213 participants enrolled into the study, 94 subjects (44.1%) were confirmed TB patients, 75 (35.2%) were human immunodeficiency virus (HIV) positive, 41 (19.2%) had advanced HIV disease (AHD), and 138 (64.8%) were HIV negative. Twenty-seven of the 213 participants were *Histoplasma* positive by antigen test and/or PCR, giving an overall prevalence rate of 12.7%. The prevalence of histoplasmosis among confirmed TB patients (7.4% [7/94]) was significantly lower than in unconfirmed TB patients (16.8% [20/119]) ($P = .04$). Participants on anti-TB therapy also had a significantly lower rate of histoplasmosis compared to those not on anti-TB drugs ($P = .00006$). The internal transcribed spacer (ITS) sequencing of the *Histoplasma* revealed a closely relatedness to *Histoplasma capsulatum*.

Conclusions. Histoplasmosis is not uncommon among presumptive TB patients. There should be proper microbiological investigation of patients presenting with symptoms suggestive of TB to exclude cases of histoplasmosis.

Keywords. histoplasmosis; HIV/AIDS; tuberculosis.

Histoplasmosis is an invasive fungal disease that occurs worldwide. It is endemic in the Ohio and Mississippi river valleys in the United States as well as Central and South America. Besides the traditional endemic regions, increasing cases are being reported from areas that were previously nonendemic for histoplasmosis including Western Africa, Southern Africa, Eastern Africa, Central Africa, and Southeast Asia, probably due to the advent of HIV/AIDS and the increased use of immunosuppressive agents [1–3]. The classical form of the disease is caused by *Histoplasma capsulatum* var *capsulatum* (Hcc) while the African type is caused by *Histoplasma capsulatum* var *duboisii* (Hcd) [1–3]. The greatest attributable risk factor for histoplasmosis is HIV/AIDS [1]. However, in the pediatric population, histoplasmosis is predominantly associated with risk factors other than HIV including environmental exposures and toxins, autoimmune diseases, childhood malignancies as well as their treatment, chronic lung diseases, immunosuppressive therapies, pancytopenia, T-cell deficiency, and malnutrition [4, 5]. The diagnosis of histoplasmosis in resource-limited settings like ours is particularly challenging because it mimics TB, a clinical entity that is very common in this region [1, 2, 5–7]. This underscores why histoplasmosis is commonly misdiagnosed as TB in our setting [1, 2, 7], with associated increased mortality, prolonged hospital stays, and unnecessary surgical interventions with attendant financial burden [2, 7, 8]. The pulmonary and disseminated forms of histoplasmosis present with features including fever, cough, generalized weakness, abdominal pain, abdominal swelling, nocturnal sweating, skin lesions, lymphadenopathy, hepatomegaly, and splenomegaly, which are also seen in all forms of TB [2, 6]. Oladele et al, in a review study that spanned 6 decades, identified major features

Received 15 June 2022; editorial decision 15 July 2022; accepted 21 July 2022; published online 25 July 2022

Correspondence: Bassey Ewa Ekeng, MBBCh, University of Calabar, Calabar, Nigeria. Diploma in Mycology (Centre of Excellence,Manchester, UK) (basseyewaekeng@gmail.com).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofac368

common to TB and disseminated histoplasmosis [DH] in the context of HIV infection including immunosuppression, pancytopenia, diarrhea, hepatomegaly, elevated liver enzymes, elevated C-reactive protein, skin lesions, and the involvement of organ systems (gastrointestinal tract, bone marrow, and central nervous system) [1]. In addition, the chest radiological findings in patients with pulmonary histoplasmosis include pulmonary infiltrates, hilar lymphadenopathy, opacities of the lung, and pleural effusion, which are also seen in TB patients [6–9]. While the knowledge and understanding of the prevalence and awareness of TB is high, not much can be said for histoplasmosis, and thus, clinical or presumptive diagnosis and treatment of TB may be rife in the current study setting, being mistaken for histoplasmosis due to similar clinical presentation of these two diseases. The question to ask is how many cases of TB or treatment failure TB could be histoplasmosis, or how many are TB coinfection with histoplasmosis, given that Nigeria is currently ranked as the sixth-highest TB-burdened country globally. This study was focused on investigating patients with suspected TB for histoplasmosis and to determine the possible risk factors associated with it.

METHODS

This was a descriptive cross-sectional study of adult patients with presumptive diagnosis of TB conducted in two major sites in Calabar, Nigeria, between April 2020 and March 2021. Calabar is located in South-South Nigeria and was the first capital of Nigeria. It comprises two local government areas: Calabar Municipality and Calabar South. One of the sites, the University of Calabar Teaching Hospital (UCTH), is a tertiary healthcare facility located in Calabar Municipality while the other, the Doctor Lawrence Henshaw Memorial Hospital, is a secondary healthcare facility located in Calabar South local government area. Both facilities have TB clinics that render specialized care to TB patients. Ethical clearance was obtained from the Health Research Ethics Committee, UCTH, and from the Cross River State Health Research Ethics Committee for this research. Written informed consent was obtained from participants before commencement of the study. Our study population falls into the priority groups for GeneXpert testing as outlined by the National TB and Leprosy Control Programme established in 1989 by the government of Nigeria to coordinate TB and leprosy control efforts in Nigeria, and consisted of patients with suspected TB regardless of their HIV status.

Inclusion and Exclusion Criteria

Inclusion criteria were (1) presentation with at least two of the following: fever, chronic cough, weight loss, hemoptysis, chest pain, cutaneous lesions, or oral ulcers; and (2) age ≥18 years. Patients receiving antifungal therapy were excluded.

Definition of Terms

Definite/confirmed TB: TB was only confirmed in a patient when sputum specimen is positive by GeneXpert [10]. Sputum culture was not done. Detection of *Mycobacterium tuberculosis* cell wall antigen, lipoarabinomannan in the urine of patients with AHD was not done.

Advanced HIV disease: Based on the World Health Organization clinical staging of HIV, participants with HIV clinical stage 3 and 4 were classified as having AHD. CD4 cell counts were not utilized in this study [11].

TB and histoplasmosis coinfection: TB and histoplasmosis coinfection were cases with confirmed TB and histoplasmosis detected either by antigen test and/or by polymerase chain reaction (PCR).

Data and Specimen Collection

Random sampling method was used for case selection. A semi-structured questionnaire was used to collect relevant sociodemographic (eg, sex, age, occupation, residential address, local government area, tribe, and level of education) and clinical data (eg, fever, cough, weight loss, central nervous system symptoms, lymphadenopathy, cutaneous lesions, oral mucosal lesions or ulcers) from participants at the point of recruitment (at the TB clinics).

Each participant was counseled on proper urine specimen collection. They were provided with a sterile screw-capped universal bottle into which they were advised to produce about 2–3 mL of midstream urine that forms specimen for laboratory detection of *Histoplasma* antigen. Samples were thereafter stored at −20 °C and processed within a period of 2 weeks, and results communicated to the managing clinicians. The study participants were also instructed to produce sputum by taking a deep inhalation and coughing in an open space with good ventilation into a wide mouthed screw-capped sputum container. All sputum specimens were promptly transported to the laboratory and stored at −80 °C.

Laboratory Procedure

All stored urine samples were allowed to thaw to room temperature prior to processing. *Histoplasma* urinary antigen test was done using the IMMY enzyme immunoassay (EIA) (Clarus *Histoplasma* GM, product reference HGM201) from IMMY (Norman, Oklahoma) following the manufacturer's instructions [12]. Concentration of *Histoplasma* antigen was calculated from the optical density (OD) of the urine samples divided by the OD of the 12.5 ng/mL standard, multiplied by 10 and reported as EIA units. Values ≥1.0 EIA unit were interpreted as positive, whereas values <1.0 EIA unit were interpreted as negative [12]. An assay was considered valid when the values of 12.5 ng/mL standard, positive control, and negative control were within

acceptable ranges as defined by the manufacturer. The positive control, negative control, and standards were included with each batch of patient specimens to provide quality assurance of the reagents [12]. Positive antigenuria were interpreted as probable cases of histoplasmosis [13]. Sputum samples collected were tested for tuberculosis using the Xpert MTB/RIF assay (Cepheid, Sunnyvale, California) and also subjected to molecular analysis by PCR for the diagnosis of histoplasmosis. Positive sputum PCR was considered as a case of proven or confirmed histoplasmosis [13]. Besides being recommended by the Centers for Disease Control and Prevention as a confirmatory diagnostic tool for histoplasmosis [13], PCR was used as the reference standard in this study for several reasons. First, the sensitivity (64.6%–87.5%) of antigen assay in the diagnosis of pulmonary histoplasmosis as shown in previous studies [14, 15] is low compared to its sensitivity of 91.3%–98% in diagnosing DH [16, 17]. Our study population consisted of patients with presumptive pulmonary TB and were more likely to have pulmonary histoplasmosis rather than DH. Second, very few studies have explored the use of antigen assay in diagnosing pulmonary histoplasmosis compared to its use in the diagnosis of DH [14, 15]. Third, in patients with acute pulmonary histoplasmosis, the sensitivity of antigen assay increases if both urine and serum are tested concurrently [14]. Our study only tested urine. The PCR procedure is documented in the Supplementary Materials [18].

Sequencing was done using the Big Dye Terminator (Sanger) kit on a 3510 ABI sequencer by Inqaba Biotechnological (Pretoria, South Africa) at a final volume of 10 µL. The components included 0.25 µL of BigDye terminator v1.1/v3.1, 2.25 µL of 5× BigDye sequencing buffer, 10 µM of forward primer (HspF) used in the PCR reaction, and 2–10 ng PCR template per 100 bp. The sequencing conditions were as follows: 32 cycles of 96 °C for 10 seconds, 55 °C for 5 seconds, and 60 °C for 4 minutes [19–21]. The sequences were edited using the bioinformatics algorithm and compared with similar sequences from the National Center for Biotechnology Information (NCBI) database using Basic Local Alignment Search Tool (BLAST). The evolutionary history was inferred using the neighbor-joining method and the evolutionary distances were computed using the Jukes-Cantor method [19–21].

Statistical Analysis

The data obtained from this study were entered and analyzed using SPSS version 22 software (SPSS Inc, Chicago, Illinois). Descriptive statistics were used to summarize the data. Mean and standard deviation were described for continuous variables, whereas categorical variables (ie, participants with histoplasmosis vs participants without histoplasmosis) were analyzed using the χ^2 test. A P value of <.05 was considered statistically significant.

RESULTS

Sociodemographic and Clinical Characteristics of Participants

Of the 213 participants, 114 (53.5%) were male, with a male-to-female ratio of 1.2:1. The mean age at presentation was 39 ± 14 years with a range of 18–81 years. The age group with the highest number of participants was 21–40 years (54.5%, n = 116), 53.1% (n = 113) of study participants had up to secondary school education level, and a significant proportion (53.5%, 114) of occupation recorded were private businesses. 181 (85.0%) participants were resident in Calabar South. Thirty-five percent (n = 75) of the subjects were people living with HIV, while 44.1% (n = 94) had confirmed TB (Table 1). Participants presented with cough (100%, n = 213), fever (91.5%, n = 195), weight loss (66.7%, n = 142), headache (18.3%, n = 39), oral mucosal lesions (1.9%, n = 4), lymphadenopathy (4.7%, n = 2), and cutaneous lesions (0.5%, n = 1).

Cases of Histoplasmosis in Participants

Of the 213 participants, 18 (8.5%) were considered to have proven and 9 (4.2%) probable histoplasmosis, giving a total prevalence of 12.7% (27/213). Fourteen cases were positive by both antigen and PCR while a total of 23 cases were positive by antigen. Of the 213 participants, 7 had both TB and histoplasmosis, giving a coinfection rate of 3.3%. The symptoms in patients with histoplasmosis (n = 27) were cough in 100% (27/27), fever in 81.5% (22/27), weight loss in 63.0% (17/27), headache in 7.4% (2/27), and oral lesions in 3.7% (1/27). None had cutaneous lesions. The frequency/rates of histoplasmosis among participants by antigen test and/or PCR were 8.0% (6/75) in HIV patients, 7.3% (3/41) in AHD patients, 7.4% (7/94) in confirmed TB patients, 16.8% (20/119) among unconfirmed TB patients, and 14.5% (20/138) among HIV-negative patients. The relationship between histoplasmosis and TB, HIV, AHD, and non-HIV patients was evaluated using the χ^2 test. The prevalence of histoplasmosis among confirmed TB participants of 7.4% (7/94) was significantly lower than 16.8% (20/119) in unconfirmed TB participants (odds ratio [OR], 0.3983 [95% confidence interval {CI}, .161–.987], $P = .04$). Participants on anti-TB therapy also had a significantly lower rate of histoplasmosis compared to those not on anti-TB drugs (OR, 0.1147 [95% CI, .033–.394], $P = .00006$), whereas there was no significant difference in the prevalence of histoplasmosis in those with and without AHD ($P = .3$; Table 1).

Evaluation of Possible Risk Factors for *Histoplasma* Infection

The risk factors considered in this study were living in a thatched house, poultry within residence, contact with hunters, location of residence close to or working at construction sites, warehouse within or close to residence, home or place of work in forested regions, proximity to a sawmill, and

Table 1. Sociodemographic and Clinical Characteristics of Participants

Characteristics	No. (%)	*Histoplasma*-Positive Participants, No. (%)	*Histoplasma*-Negative Participants, No. (%)	*P* Value
All participants	213	27 (12.7)	186 (87.3)	
Age group (years)				
<21	14 (6.6)	1 (7.1)	13 (92.9)	.9
21–40	116 (54.5)	14 (12.1)	102 (87.9)	
41–60	66 (31.0)	9 (13.6)	57 (90.5)	
>60	17 (7.9)	3 (17.6)	14 (82.4)	
Sex				
Male	114 (53.5)	18 (15.7)	96 (84.2)	.1
Female	99 (46.5)	9 (9.1)	90 (90.9)	
Occupational status				
Unemployed	70 (32.9)	7 (10.0)	63 (90.0)	.6
Private business	114 (53.5)	15 (13.2)	99 (86.8)	
Professionals	29 (13.6)	5 (17.2)	24 (82.8)	
Educational level				
Primary	28 (13.1)	3 (10.7)	25 (89.3)	.9
Secondary	113 (53.1)	13 (11.5)	100 (88.5)	
Tertiary	66 (30.9)	10 (15.2)	56 (84.8)	
No formal education	6 (2.8)	1 (16.7)	5 (83.3)	
Residence (LGA)				
Calabar South	181 (85)	22 (12.2)	159 (87.8)	.4
Calabar Municipality	20 (9.4)	2 (10.0)	18 (90.0)	
Akpabuyo	5 (2.3)	1 (20.0)	4 (80.0)	
Akampka	1 (0.5)	…	…	
Odukpani	3 (1.4)	1 (33.3)	2 (66.7)	
Ogoja	1 (0.5)	…	…	
Uyo	2 (0.9)	1 (50.0)	1 (50.0)	
HIV status				
Positive	75 (35.2)	6 (8.0)	69 (92.0)	.1
Negative	138 (64.8)	21 (15.2)	117 (84.8)	
Advanced HIV disease				
Yes	41 (19.2)	3 (7.3)	38 (92.7)	.3
No	172 (80.8)	24 (14.0)	148 (86.0)	
Tuberculosis				
Confirmed	94 (44.1)	7 (7.4)	87 (92.6)	.04[a]
Unconfirmed	119 (55.9)	20 (16.8)	99 (83.2)	
Anti-TB therapy				
Yes	100 (46.9)	3 (3.0)	97 (97.0)	.00006[b]
No	113 (53.1)	24 (21.2)	89 (78.8)	

Abbreviations: HIV, human immunodeficiency virus, LGA, Local Government Area; TB, tuberculosis.

[a]Statistically significant (odds ratio, 0.3983 [95% confidence interval, .161–.987]).

[b]Statistically significant (odds ratio, 0.1147 [95% confidence interval, .033–.394]).

history of travel to an endemic region. Of the 27, 59.3% (16/27) were exposed to all or some of the above stated risk factors. However, the association was not statistically significant (Table 2). The association between age ($P = .9$), sex ($P = .1$), occupation ($P = .6$), educational level ($P = .9$), residence ($P = .4$), and *Histoplasma* infection was also not statistically significant (Table 1).

Concordance Between *Histoplasma* Urinary Antigen Test and Sputum PCR

Compared with sputum PCR, the diagnosis of histoplasmosis in this study by detection of *Histoplasma* urinary antigen was observed to be statistically significant (Table 3). Using sputum PCR as a reference standard for diagnosis [13], the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of *Histoplasma* urinary antigen test are as represented in Table 4.

Molecular Characterization

The obtained internal transcribed spacer (ITS) sequence from the isolates (Figure 1) produced an exact match during the megablast search for highly similar sequences from the NCBI nonredundant nucleotide (nr/nt) database. The ITS of the isolates showed a percentage similarity to other species at 99%–100%. The evolutionary distances computed using

Table 2. Evaluation of Risk Factors for *Histoplasma* Infection

Risk Factor	No. (%)	*Histoplasma*-Positive Participants, No. (%)	*Histoplasma*-Negative Participants, No. (%)	*P* Value
All participants	213	27 (12.7)	186 (87.3)	
Living in a thatched house				
No	200 (93.9)	25 (12.5)	175 (87.5)	.8
Yes	13 (6.1)	2 (15.4)	11 (84.6)	
Farming/poultry within residence				
No	144 (67.6)	17 (11.8)	127 (88.2)	.6
Yes	69 (32.4)	10 (14.5)	59 (85.5)	
Contact with hunters				
No	204 (95.8)	25 (12.3)	179 (87.7)	.4
Yes	9 (4.2)	2 (22.2)	7 (77.8)	
Proximity to construction sites				
No	168 (78.9)	18 (10.7)	150 (89.3)	.09
Yes	45 (21.1)	9 (20.0)	36 (80.0)	
Residence close to a warehouse				
No	196 (92.0)	24 (12.2)	172 (87.8)	.5
Yes	17 (8.0)	3 (17.6)	14 (82.4)	

the Jukes-Cantor method agreed with the phylogenetic placement of the isolates within *Histoplasma* species and revealed a closely relatedness to *Histoplasma capsulatum* than other species (Supplementary Figures 1–3). Only 3 (MW439314.1, MW504636.1, MW492387.1) of the sequenced amplicons were uploaded in the gene bank to avoid duplication as most of the sequences were identical.

DISCUSSION

The prevalence of histoplasmosis in this study was 12.7% (27/213). This is relatively high and quite alarming as histoplasmosis was not considered as a differential by the attending clinicians. This finding was much higher than what was documented from northern Tanzania in febrile patients that gave a rate of 0.9% (9/970) [22]. The low prevalence from northern Tanzania could have been from a number of factors. First, the study was not focused on a targeted population. Second, a review of their methodology revealed that samples were pooled and tests were run 6–18 months, contrary to manufacturers' instructions of a maximum of about 2 months, which may have accounted for false-negative values.

Prior to the advent of HIV/AIDS, the occurrence of DH was low and limited to patients with hematological cancers, immunosuppression due to chemotherapy, primary cellular immunodeficiencies, diabetes mellitus, or advanced age, or in children <2 years of age [23]. Currently, the estimated incidence of DH varies from 5% to 25% in the AHD population [24]. In Africa, a review that spanned 6 decades (1952–2017) by Oladele et al revealed 470 cases of histoplasmosis, of which 38% (178) of the cases were in HIV patients [1]. In this study, the prevalence of histoplasmosis in the AHD population was 7.3% (3/41). This finding was lower than reports from other studies done in Africa and other regions, probably because our study was not focused on people living with HIV/AIDS [25–28]. A recent report from Cameroon revealed a 26% (36/138) prevalence in the AHD population using urinary antigen test [25]. An earlier report in 2015 from Cameroon reported a prevalence of 13% (7/56) in the AHD population [26]. Reports from Latin America also revealed high prevalence rates, with 22% (123/570) prevalence reported from Brazil, while another

Table 3. Concordance Between *Histoplasma* Urinary Antigen Test and Sputum Polymerase Chain Reaction

	Histoplasma Antigen Assay			
Sputum PCR	Negative	Positive	Total	Correlation
Negative	186	9	195	$\chi^2 = 91.6$
Positive	4	14	18	$P < .00001$
Total	190	23	213	

Abbreviation: PCR, polymerase chain reaction.

Table 4. Sensitivity, Specificity, Accuracy, and Predictive Values of *Histoplasma* Antigen Test

Test characteristics	Formula	Value
Sensitivity	TP/TP + FN	77.8%
Specificity	TN/TN + FP	95.4%
PPV	TP/TP + FP	60.9%
NPV	TN/TN + FN	97.9%
Accuracy	TP + TN/FP + FN	93.9%
PLR	(Sensitivity) / (1 – specificity)	16.9%
NLR	(1 – sensitivity) / (specificity)	0.2

Abbreviations: FN, false negative; FP, false positive; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; TN, true negative; TP, true positive.

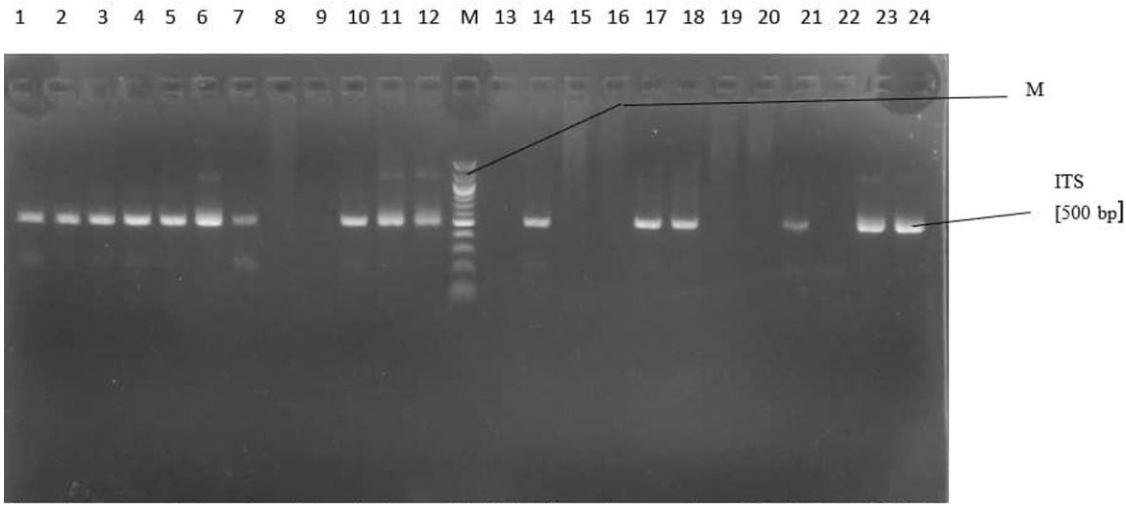

Figure 1. Agarose gel electrophoresis showing the amplified internal transcribed spacer (ITS) of *Histoplasma* species. Lanes 1–7, 10–12, 14, 17, 18, 21, 23, and 24 represent the ITS bands at 500 bp, whereas lane M represents the 100-bp molecular ladder.

study from Mexico in the same population revealed a prevalence of 30% (85/228) [27, 28].

The histoplasmosis and TB coinfection rate in this study was 3.3% (7/213). Data on histoplasmosis and TB coinfection in Africa are limited. However, case reports have been documented across all ages [2, 7].

A high prevalence (20/138 [14.5%]) of histoplasmosis was also observed in the non-HIV population. This emphasizes the need to also investigate immunocompetent patients presenting with symptoms suggestive of TB for histoplasmosis besides patients living with HIV/AIDS. One of the challenges in our setting is the poor index of suspicion on the part of clinicians, which causes patients to be placed on anti-TB therapy despite being GeneXpert or acid-fast bacilli negative [2, 6]. This is further corroborated by the finding from this study, which showed higher prevalence of histoplasmosis in unconfirmed TB patients (16.8%) than in confirmed TB patients (7.4%). These unconfirmed TB cases must have been misdiagnosed as TB when they were actually cases of histoplasmosis. The odds of such misdiagnosis are less (OR, 0.3983) with confirmed TB than unconfirmed TB cases, which strongly supports the need to always confirm TB with GeneXpert or sputum smear microscopy before initiating anti-TB therapy. In addition, besides screening for histoplasmosis, there is need for a wider range of investigations for patients presenting with signs and symptoms of TB. In this study, 83.2% (99/119) of participants with unconfirmed TB (n = 119) were negative for antigen and PCR and may have presented with clinical conditions other than TB and histoplasmosis. Moreover, Gene Xpert may have missed cases of culture proven TB as well as TB detected by lipoarabinomannan assay among participants with unconfirmed TB.

Looking at sociodemographic characteristics, males were observed to have higher rates of histoplasmosis (n = 18/114 [15.8%]). Although the rate appears higher in males, it was not statistically significant ($P = .15$). The higher rates in males might likely be due to the preponderance of male participants. A similar finding was reported in a systemic review from Brazil, which documented a rate of 78% (male-to-female ratio of 4:1) in males [29]. With regards to the occupation of participants, a significant proportion including those with private businesses and professionals were low risk for histoplasmosis and as such did not affect the rates of *Histoplasma* infection in this study.

Concerning sensitivity, specificity, PPV, NPV, accuracy, positive likelihood ratio, and negative likelihood ratio of *Histoplasma* antigen test in this study, we were not keened to compare values with findings from other studies, because the standards used in those studies was not PCR [14–17]. Compared with sputum PCR, *Histoplasma* urinary antigen test is more feasible in our setting for the following reasons: (1) It is cheaper, quicker (turnaround time of 3–5 hours), and needs less expertise to perform; (2) sputum samples analyzed in this study were pooled and processed at the end of the study, with DNA being denatured in some of the samples; (3) although molecular assays gave excellent analytical performance, there is no consensus on PCR protocols and gene targets, which results in varying sensitivity and specificity patterns; and (4) PCR machines are not readily available in routine laboratories, especially in resource-limited settings [1, 6]. *Histoplasma* urinary antigen test should therefore be deployed for the diagnosis of histoplasmosis in resource-limited settings like ours where sputum PCR is not a routine.

Contrary to the findings from a previous review that Hcd is predominant in Western Africa [1], all sequenced amplicons in this study showed a closely relatedness to Hcc than other species. Hcc causes classical histoplasmosis, which predominantly presents with pulmonary symptoms. Similarly, the predominant symptom among participants diagnosed with histoplasmosis was cough (100%, n = 27). Extrapulmonary manifestations, including cutaneous ulcers associated with African histoplasmosis [1], were not seen in any of the participants positive for *Histoplasma* infection.

CONCLUSIONS

This study reports a high prevalence of histoplasmosis among presumptive TB patients and coinfections of TB and histoplasmosis. It is imperative that histoplasmosis screening be included in the protocol for the management of the "at risk" population.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. B. E. E. was involved in the conception and design of the study, literature review, data analysis, writing of the manuscript, review, and editing. R. O. O., U. E. E., and E. A. O. were involved in the conception and design of the study, participated in writing the manuscript, and conducted a critical review of the manuscript. T. M. was involved in the laboratory procedure, participated in writing the manuscript, and conducted a critical review of the manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgments. We thank IMMY for donating the *Histoplasma* antigen enzyme-linked immunosorbent assay kits that were used in this study.

Potential conflicts of interest. All authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Oladele RO, Ayanlowo OO, Richardson MD, Denning DW. Histoplasmosis in Africa: an emerging or a neglected disease? PLoS Negl Trop Dis **2018**; 12: e0006046.
2. Ekeng BE, Edem K, Akintan P, Oladele R. Histoplasmosis in African children: clinical features, diagnosis and treatment. Ther Adv Infect Dis **2022**; 9:1–16.
3. Ashraf N, Kubat RC, Poplin V, et al. Re-drawing the maps for endemic mycoses. Mycopathologia **2020**; 185:843–65.
4. MacInnes R, Warris A. Paediatric histoplasmosis 2000–2019: a review of 83 cases. J Fungi **2021**; 7:448.
5. Ekeng BE, Edem K, Amamilo I, Panos Z, Denning DW, Oladele RO. Histoplasmosis in children; HIV/AIDS not a major driver. J Fungi **2021**; 7:530.
6. Ekeng BE, Davies AA, Osaigbovo II, Warris A, Oladele RO, Denning DW. Pulmonary and extrapulmonary manifestations of fungal infections misdiagnosed as tuberculosis: the need for prompt diagnosis and management. J Fungi **2022**; 8:460.
7. Mandengue CE, Ekeng BE, Oladele RO. Disseminated histoplasmosis; a threat in advanced HIV disease population in sub-Saharan Africa? J Adv Med **2021**; 33: 115–44.
8. Ewa AU, Ekeng BE, Bassey GE, Akpah EU, Aiyudubie AO, Nweke LN. Disseminated histoplasmosis in a 17-year-old Nigerian male patient: a case report. Asian Pac J Trop Med **2022**; 15:283–6.
9. Kauffman CA. Histoplasmosis: a clinical and laboratory update. Clin Microbiol Rev **2007**; 20:115–32.
10. World Health Organization (WHO). Guidance for national tuberculosis programmes on the management of tuberculosis in children. 2nd ed. Annex 2. TB and treatment outcome definitions. Geneva, Switzerland: WHO; **2014**.
11. Weinberg JL, Kovarik L. The WHO clinical staging system for HIV/AIDS. AMA J Ethics **2010**; 12:202–6.
12. IMMY. Clarus *Histoplasma* GM enzyme immunoassay. Norman, OK: IMMY, Inc; **2018**.
13. Centers for Disease Control and Prevention. National Notifiable Diseases Surveillance System. https://ndc.services.cdc.gov/case-definitions/histoplasmosis-2017/. Accessed April 16, 2021.
14. Swartzentruber S, Rhodes L, Kurkjian K, et al. Diagnosis of acute pulmonary histoplasmosis by antigen detection. Clin Infect Dis **2009**; 49:1878–82.
15. Hage CA, Ribes JA, Wengenack NL, et al. A multicenter evaluation of tests for diagnosis of histoplasmosis. Clin Infect Dis **2011**; 53:448–54.
16. Cáceres DH, Samayoa BE, Medina NG, et al. Multi center validation of commercial antigenuria reagents to diagnose progressive disseminated histoplasmosis in people living with HIV/AIDS in two Latin American countries. J Clin Microbiol **2018**; 56:e01959-17.
17. Martınez-Gamboa A, Niembro-Ortega MD, Torres-Gonzalez P, et al. Diagnostic accuracy of antigen detection in urine and molecular assays testing in different clinical samples for the diagnosis of progressive disseminated histoplasmosis in patients living with HIV/AIDS: a prospective multicenter study in Mexico. PLoS Negl Trop Dis **2021**; 15:e0009215.
18. Bracca A, Tosello ME, Girardini JE, Amigot SL, Gomez C, Serra E. Molecular detection of *Histoplasma capsulatum* var capsulatum in human clinical samples. J Clin Microbiol **2003**; 41:1753–5.
19. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol **1987**; 4:406–25.
20. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **1985**; 39:783–91.
21. Jukes TH, Cantor CR. Evolution of protein molecules. In: Munro HN, ed. Mammalian Protein Metabolism. New York: Academic Press, **1969**:21–132.
22. Lofgrena SM, Kirsch EJ, Maro VP, et al. Histoplasmosis among hospitalized febrile patients in northern Tanzania. Trans R Soc Trop Med Hygiene **2012**; 106: 504–7.
23. Myint T, Leedy N, Cari EV, Wheat LJ. HIV-associated histoplasmosis: current perspectives. HIV AIDS **2020**; 12:113–25.
24. Silva TC, Tremea CM, Zara ALSA, et al. Prevalence and lethality among patients with histoplasmosis and AIDS in the midwest of Brazil. Mycoses **2017**; 60:59–65.
25. Kuate MPN, Nyasa R, Mandengue C, Tendongfor N, Bongomin F, Denning DW. Screening for acute disseminated histoplasmosis in HIV disease using urinary antigen detection enzyme immunoassay: a pilot study in Cameroon. JMicrobiol Methods **2021**; 185:106226.
26. Mandengue CE, Ngandjio A, Atangana PJA. Histoplasmosis in HIV-infected persons, Yaoundé, Cameroon. Emerg Infect Dis **2015**; 21:2094–6.
27. Falci DR, Monteiro AA, Braz Caurio CF, Magalhães TCO, Xavier MO. Histoplasmosis, an underdiagnosed disease affecting people living with HIV/AIDS in Brazil: results of a multicentre prospective cohort study using both classical mycology tests and *Histoplasma* urine antigen detection. Open Forum Infect Dis **2019**; 6:ofzo73.
28. Torres-Gonzalez P, Niembro-Ortega MD, Martınez-Gamboa A, et al. Diagnostic accuracy cohort study and clinical value of the *Histoplasma* urine antigen (ALPHA *Histoplasma* EIA) for disseminated histoplasmosis among HIV infected patients: a multicenter study. PLoS Negl Trop Dis **2018**; 12:e0006872.
29. Almeida M, Almeida-Silva F, Guimaraes AJ, Almeida-Paes R, Zancope-Oliveira RM. The occurrence of histoplasmosis in Brazil: a systematic review. Int J Infect Dis **2019**; 86:147–56.