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## Original article

# Prevalence of disseminated *Histoplasma capsulatum* in pulmonary infection patients, attending National Tuberculosis and Leprosy Training Centre, Saye – Nigeria

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## ABSTRACT

**Background:** Histoplasmosis is a fungus disease caused by a dimorphic organism called *Histoplasma capsulatum*, it is endemic in United States, Asia, and Africa. Inoculation is through inhalation of infectious spores. Histoplasmosis is missed in diagnosis because it mimics tuberculosis in clinical presentations and not all patients suffering from tuberculosis are tuberculosis patients. The aim is to determine the prevalence of disseminated histoplasmosis among patients with pulmonary infections. **Method:** This is cross sectional and hospital based. Samples were collected from patients and questionnaires were administered. Three hundred and twenty-two (322) urine samples were aseptically collected in urine tubes and analyzed by enzyme linked immunosorbent assay to detect the presence of galactomannan in the urine samples. **Results:** Out of 322 samples analyzed, 12 (3.74 %) were positive for the presence of galactomannan while 310 (96.27 %) were negative. Participants of the geriatric age group had the highest prevalence of 12.5 %. Male 8.07 % were more prone to histoplasmosis disease than their female 3.10 % counterpart. The married displayed a high prevalence value of 6.25 % than singles 2.25 % and the divorced 3.57 % and framer were demonstrated a high value of 7.69 % prevalence. Among the risk factors trader had an odd ratio of 3.800 and chest discomfort 1.037 % is a disease symptom associated with histoplasmosis. **Conclusion:** Disseminated histoplasmosis occur among the study participants through low in prevalence, awareness of its presence should be known to the general public and clinician, diagnosis of histoplasmosis is be done along that of Tuberculosis because it can be co-infection with Tuberculosis for proper treatment regime.

## Introduction

Histoplasmosis is a systemic disease caused by the fungus *Histoplasma capsulatum* (*H. capsulatum*) [1]. The disease is also called cave disease, Darling disease and Ohio valley disease. Human histoplasmosis is due to two varieties of the pathogen, *H. capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii*, the former being the causative agent of classical histoplasmosis [2] while the latter is the etiologic cause for African histoplasmosis [3]. This disease is highly endemic in some regions of North America, Central America, and South America and is also reported in certain countries of Asia and Africa including Nigeria.

## Pathogenesis

Generally, the environmental conditions present in areas of high endemicity are a moderate climate with constant humidity [4]. It is a saprophytic mold in the soil and grows at 25 °C. Human infection occurs as a result of inhalation of infectious spores which are deposited in the alveoli and conversion to a parasitic yeast form in tissues of immunocompromised patients (with disabled cellular immunity), while in the immunocompetent host primary infections are asymptomatic or occur as a self-limiting flu-like illness. This germination and conversion can occur prior to or after ingestion by pulmonary macrophages [5]. Conidia and yeasts are ingested by macrophages and reticuloendothelial cells where the organisms can survive within phagolysosomes [6]. Once within the macrophage, the yeast multiplies and travel to hilar and mediastinal lymph nodes where they gain access to the blood circulation and disseminate to organs [7]. People affected are those with impaired immunity, including those living with HIV, among whom the most frequent clinical presentation is disseminated histoplasmosis. Disseminated histoplasmosis symptoms are non-specific and can be indistinguishable from those of other infectious diseases, especially disseminated tuberculosis (TB), hence, complicating diagnosis and treatment [8]. The most frequent opportunistic infections caused by fungal pathogens among people living with HIV in the Americas is histoplasmosis and can be responsible for 13 – 48 % of AIDS-related deaths every year in this Region [9]. Disseminated histoplasmosis usually leads to death within a few weeks if untreated. This research is hospital based and aimed to determine the prevalence of disseminated histoplasmosis in patients with pulmonary infection, Saye – Nigeria

## Predisposing factors

Different conditions predispose an individual to disseminated histoplasmosis, of which are immunocompromised condition (example, acquired immune deficiency syndrome (AIDS)), taking of immunosuppressants therapy (these includes, glucocorticoids, anti-rejection therapies in solid organ transplantation, or TNF- $\alpha$  inhibitor therapies, cancer patients), primary immunodeficiency, and people of geriatric age [5].

## Objectives

The specific objective is to determine the reason while patients diagnosed of *Mycobacterium tuberculosis* are often not responding to treated after undergoing the normal treatment of tuberculosis infection in National Tuberculosis and Leprosy Training Centre, Saye – Nigeria

## Explanation for the scientific background and the rationale

Histoplasmosis is a dimorphic fungi disease caused by *H. capsulatum*. The pathogenic yeast form can either be localized in the lungs or disseminated to other organs in the body. It is burden among patients suffering from TB in Nigeria, hence increased in death rate recorded in Tb patients. It mimics TB in clinical presentations and hence missed in diagnosis. However, due to its similarities in signs and symptoms, it can exist in a patient as *H. capsulatum* alone or as a missed infection with a *Mycobacterium tuberculosis* patient. This problem triggered me to embark on this research, while patients with Tb are dying in Northern Nigeria after undergoing a full TB treatment. Therefore, *Mycobacterium tuberculosis* patients should be properly screened for *Histoplasma capsulatum* before the commencement of therapy, as the right diagnosis will lead the clinicians to adequate treatment regime.

## Material and methods

### Study area

This study was conducted in Zaria, Nigeria. Zaria is located between latitudes 11°04'00"N and 11°08' 800"N and Longitude 7°36 ' 00"E and 7°44 ' 00"E [10]. The total area covered is about 2638.20 Km<sup>2</sup> with an altitude of about 615 meters above sea level [11]. The study area consists of one distinct settlement, Saye in Zaria metropolis. The main occupation of Zaria people is farming. They also engage in trading and some are civil servants.

**Study design**

This is a cross sectional and hospital based. Samples were collected from patients and questionnaires were administered.

**Ethical clearance**

Ethical clearance (NBTL/TR6/ZA/182/ve13v) for the study was obtained from the Ethical Committees of National Tuberculosis and Leprosy Training and Referral Hospital Saye, Nigeria. Similarly, informed consent was obtained from each of the participants prior to recruitment into the study.

**Study population**

This comprised patients with signs and symptoms of pulmonary infection attending a selected hospital, Saye - Nigeria

**Inclusion criteria**

Patients presenting with pulmonary diseases who gave consent.

**Exclusion criteria**

Non-pulmonary disease patients and those patients who did not give consent.

**Hypotheses**

**H<sub>0</sub>** = Pulmonary disease Patients do not have histoplasmosis disease

**H<sub>a</sub>** = Pulmonary disease Patients have histoplasmosis disease

**Administration of questionnaires**

A structured questionnaire was used to obtain data on socio-demographic characteristics and risk factors associated with *H. capsulatum* infection from consenting participants. Risk factors included immunocompromise, construction work, renovation of abandoned building, farming and gardening.

**Diagnostic detection / collection of urine sample**

Diagnostic methods like, antigen detection, cultures, serology, or direct microscopy can be used to diagnoses histoplasmosis. However, in this research antigen detection method was endorsed.

Urine samples were collected in a universal container from pulmonary disease patients attending National Tuberculosis and Leprosy Training and Referral Hospital Saye, Zaria, Nigeria. Patients were educated by a qualified Medical Laboratory Personnel on how to collect midstream urine. A total of 322 urine samples were collected.

**Preparation of urine sample**

Urine for ELISA assay should not be frozen and if, it should be allowed to thorn to room temperature

before use. The urine used in this study was a freshly collected and undiluted nor centrifuged

**Detection of *Histoplasma* antigen in urine samples using enzyme linked immunosorbent assay (ELISA)**

Urine samples (no 322) was collected prospectively from pulmonary infection patients attending National tuberculosis and Leprosy Hospital Saye between December, 2018 to March 2019 and submitted for routine clinical evaluation with IMMY Galactomannan (GM) EIA in Microbiology Laboratory, for detection of *Histoplasma* GM. Qualitative and quantitative assay were carried out. The IMMY GM EIA is a quantitative, antigen capture EIA for detection of *H. capsulatum* GM. A Testing was performed on the Triturus automated EIA analyzer (Grifols, Miami, FL).

Procedure: A 100 µl of aseptically collected urine was added to each micro well plate containing antihistiplasma monoclonal IgG antibody (capture antibody) and incubated at 37 °C for 60 minutes. Using a pipette, content from each well was aspirated and discarded into a biohazard receptacle. Wells were filled with 300 µl 1X Wash Buffer using a multichannel pipette and plates content were damped followed by the addition of 100 µl of the horse-reddish peroxidase conjugated anti-*Histoplasma* IgG monoclonal antibody (Detect Antibody) and incubation of the plates at 37 °C for 45 minutes. The contents were aspirated out and washed thoroughly and damped. A 100 µl of the tetramethylbenzidine (TMB) substrate was added to each well and mixed by gently shaking 5 seconds on countertop. Plates were incubated at 37 °C for 30 minutes. After that, 100 µl of Methane sulfonic acid (Stop Solution) was added to each well and the deep yellow color changes to blue color.

Readings were taken within 15 minutes of test completion and an optical density (absorbance) was determined with a microplate reader at 450 nm and a reference wave length of 630 nm was used [12].

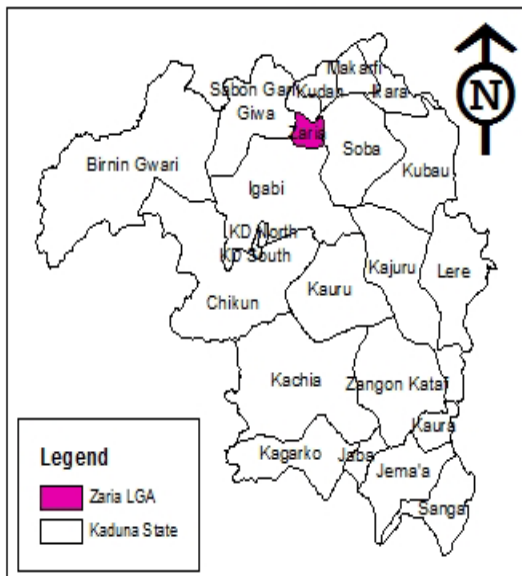
A standard curve was generated with each run by using seven calibrators, i.e., a 0.0-ng/ml calibrator (wash buffer) was added to the seven calibrators recommended by the manufacturer (0.4, 0.8, 1.6, 3.1, 6.25, 12.5, and 25 ng/ml). OD values for each of the eight calibrators were plotted using a linear regression curve, and quantitative patient results (in ng/ml) were calculated by mapping the sample OD value against the standard curve.

An assay is considered valid, when the standard fall within the acceptable, blanked OD range and  $R^2 \geq 0.990$  using the four-parameter curve-fit.

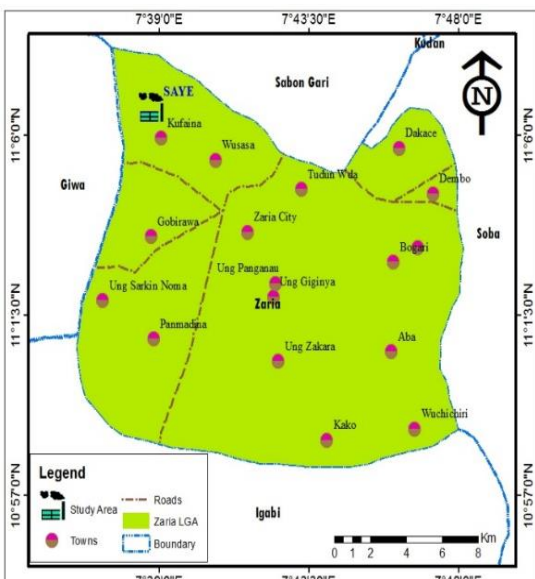
**Data analysis**

Data were analyzed using statistical package for social sciences (SPSS) 22.0 computer software package and 5% significant level was used. Chi square was used to test for significant difference while odd ratio was used to test for association between risk factor and disease condition. In all the analysis,  $p \leq 0.05$  was taken to be statistically significant.

**Figure 1.** Map of Kaduna showing Zaria where the study area is located.



**Figure 2.** Map of Zaria showing Saye where the study was carried out.



**Results**

**Prevalence of *Histoplasma capsulatum* infection among the study participants**

The result for detection of *Histoplasma* antigen in urine samples using enzyme linked immunosorbent assay (ELISA) shows that 12 samples were positive out of 322 giving a prevalence of 3.74 % as shown in **figure (3)**.

**Sociodemographic factors associated with histoplasmosis among the study participants**

**Table 1** shows the result of urine prevalence of Histoplasmosis among the study participants based on their sociodemographic factors. Considering the factor of age, participants of the age group 80 and above recorded higher prevalence of 12.5 % while those of age group 50 - 59 years had the lowest prevalence of 2.32 % although the difference was statistically significant.

According to gender, males had a higher prevalence of 8.07 % than females 3.10 % with statistically significant differences ( $p = 0.00$  %).

Based on the marital status, those who were married recorded the highest prevalence 6.25 % difference was statistically significant ( $p = 0.00$  %).

As for the factor of occupation, farming recorded the highest prevalence of 7.69 %, the difference was statistically significant ( $p < 0.05$ ).

**Risk factors associated with histoplasmosis among the study participants based on culture**

As shown in **table (2)**, participants belonging to the group trading among the risk factors considered in the study has an odd ratio of 3.800, which is a factor associated with histoplasmosis

**Clinical features of the study participants associated with histoplasmosis**

Of all the symptoms of histoplasmosis considered in this study Chest discomfort is a symptom associated with the disease among the study participants. The result is as presented in **table (3)** below.

**Table 1.** Prevalence of histoplasmosis in relation to sociodemographic factors of the study participants.

Age	Number Examined	positive (%)	Df	$\chi^2$	P-value
10-19	31	1(3.22)	7	97.975	0.00
20-29	74	1(5.40)			
30-39	72	4(8.33)			
40-49	48	2(6.25)			
50-59	43	2(2.32)			
60-69	25	0(4.00)			
70-79	21	1(4.76)			
80-above	8	1(12.5)			
	<b>322</b>	<b>12(3.73)</b>			
<b>Gender</b>					
Male	161	7(8.07)	1	0.00	1.00
Female	161	5(3.10)			
	<b>322</b>	<b>12(3.73)</b>			
<b>Marital Status</b>					
Married	224	11(6.25)	2	198.44	0.00
Single	70	0(4.28)			
Divorced	28	1(3.57)			
	<b>322</b>	<b>12(3.73)</b>			
<b>Occupation</b>					
Self employed	157	5 (3.18)	3	116.91	0.00
Unemployed	73	2(2.59)			
Civil Servant	23	0(0.00)			
Farming	69	5(7.69)			
	<b>322</b>	<b>12(3.73)</b>			

**Table 2.** Prevalence of histoplasmosis among the study participants based on risk factors considered in the study.

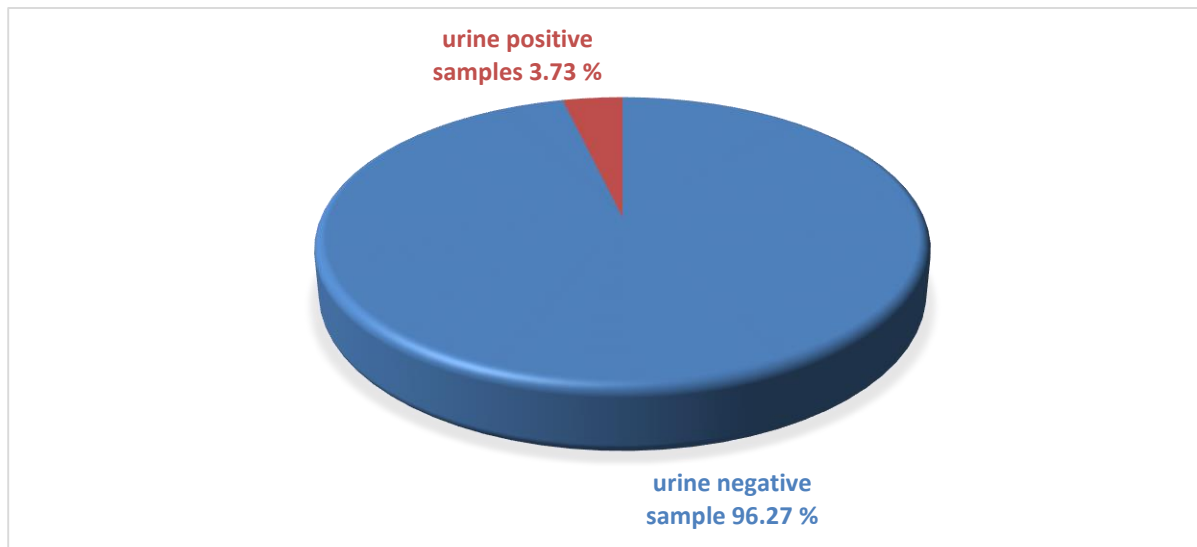
Variables	Option	No Examined	No Positive (%)	Odds ratio	Lower limit	Upper limit
<b>Immuno Compromised</b>	Yes	1	0(0.00)	0.950	0.938	0.981
	No	321	12(3.74)			
<b>Construction Worker</b>	Yes	2	0(0.00)	0.959	0.938	0.981
	No	320	12(3.76)			
<b>Farming</b>	Yes	97	7(7.22)	0.925	0.283	3.024
	No	225	5(2.22)			
<b>Traders</b>	Yes	4	0(0.00)	3.800	1.931	4.332
	No	318	12(3.77)			
<b>Others</b>	Yes	218	7(3.21)	2.452	0.527	5.398
	No	104	5(4.81)			

**Key:** Others = No specific work or trade engaged/ chest discom. = chest discomfort

**Table 3.** Prevalence of histoplasmosis in relation to clinical presentation among the study participants.

Variables	Option	No. Examined	No Positive (%)	Odd ratio	Lower limit	Upper limit
<b>Fever</b>	Yes	217	8(3.69)	0.766	0.244	2.400
	No	105	4(3.81)			
<b>Chill</b>	Yes	93	1(1.08)	1.242	0.365	4.337
	No	229	11(4.80)			
<b>Headache</b>	Yes	222	7(3.15)	1.242	0.362	5.158
	No	100	5(5.00)			
<b>Chest discom.</b>	Yes	310	11(3.55)	1.037	1.015	1.059
	No	12	1(8.33)			
<b>Muscle ache</b>	Yes	210	6(2.86)	0.738	0.229	2.381
	No	112	6(5.36)			
<b>Sleeplessness</b>	Yes	27	1(3.70)	0.993	0.123	7.997
	No	295	11(3.72)			

Key: Chest discom. = chest discomfort

**Figure 3.** Prevalence of *Histoplasma capsulatum* among pulmonary infection patients using ELISA.

### Discussion

In this study, only a few percent of individuals experienced disseminated form of histoplasmosis which is detected by ELISA in the urine sample. A low prevalence of ELISA positive urine sample 1(0.31%) was recorded, this shows that only very few cases of disseminated histoplasmosis were seen in our study population. This might be as a result of patients developing cellular immunity which eradicate the infection, as is supported by **Shojaei et al.** [13] and **Choi et al.** [14] who reported in their study that dissemination is usually

nonprogressive and can be resolved even without therapy upon the development of cellular immunity to *H. capsulatum*.

The prevalence of disseminated histoplasmosis in relation to age among the study participants, the geriatric age group 80 – 89 years had the highest prevalence, which is significant to disease formation. This may be as a result of the waning immunity of the elderly which is a risk factor for *Histoplasma* infection. This view is supported by **Shojaei et al.** [13], who reported that people develop histoplasmosis as they grow older. It

may also be due to reactivation of their initial primary infection as a result of waning immunity [15].

The distribution of *H. capsulatum* infection across gender showed that males (8.07 %) had a higher prevalence than the female (3.10 %) though not statistically significant. Literature has it that the males are more susceptible to systemic infections, of which histoplasmosis is one, probably due to the fact that they engage more in outdoor activities that predispose them to infectious spores of *Histoplasma capsulatum* infection such as construction work, demolishing of old building [3,6].

*Histoplasma capsulatum* infection differed across the marital status of the participants with the married being more infected ( $p < 0.000$ ), this may be due to the fact that some married women engage in outdoor activities such as farming in order to augment the spouses' income, which may predispose them to *Histoplasma* infection. Also, the trauma suffered by some married women who are physically abused by their spouses has been reported to lower their immunity making them prone to opportunistic infection such as *Histoplasma* infection [3, 13].

Across occupation of the study participants, the distribution showed that farmer 7.69 % had a higher prevalence. As Farmers their job nature exposes them to infectious spore of *H. capsulatum* such as poultry keeping and farming on a soil that contain bats and birds dropping or bird's droppings used as manure may pose a risk for *H. capsulatum* infection. This is similar to the observation documented by **Ekeng et al.** [16] and **Coffey et al.** [17] who reported that soil contaminated with bids / bats droppings is a factor for acquiring histoplasmosis.

Of the risk factors considered in the study, trading (3.800) was associated with disseminated histoplasmosis, this may be due to immunodepression of the individual hence exposure to opportunistic infection. This is supported by **Myint et al.** [18] who in his study stated that immunologic factor and absence of antiretroviral treatment leads to acquisition of opportunistic infections. **Islam et al.** [19] reported that histoplasmosis is tightly connected with a dysfunctional immune system particularly conditions with compromised cellular immunity affecting T-cell are prone to the propagation of the diseases, a scenario often resembling miliary

tuberculosis. It is equally possible, that patients might have been exposed to fomites contaminated with the conidia of *H. capsulatum* in the environment from which they contracted the disease. **Nega et al.** [20] and **Avasthi et al.** [21] have reported that fomites contaminated with the conidia of *H. capsulatum* can cause any infection if an individual is exposed.

Chest discomfort (1.037) is a symptom associated with histoplasmosis in the study, this goes with the literature that headache, chest pain, fever, chill is the most common symptom of histoplasmosis [13, 22].

### Conclusion

An overall prevalence of 3.73 % for the disseminated histoplasmosis was obtained among the study participants, confirming the presence of the disease in Northern part of the Nigeria.

Farmers and married participants were the demographic factors that were strongly associated with histoplasmosis in our study, while age and gender were not statistically associated with histoplasmosis.

### Recommendation

Patients presenting symptoms of pulmonary infection similar to tuberculosis should be screened for histoplasmosis with which it shares symptoms.

Study with wider coverage should be undertaken to assess the national distribution of histoplasmosis since no much data is available as such.

As a re-emerging disease in Nigeria, histoplasmosis should be included in awareness campaign and implementation of surveillance program with a view of controlling and preventing its spread.

World Health Organization (WHO) has provided guidelines which aim to provide recommendations for the diagnosis, treatment, and management of disseminated histoplasmosis in persons living with HIV. Although the burden of disease is concentrated in the Americas, the recommendations contained within these guidelines are applicable globally. These guidelines were produced in accordance with (WHO) handbook for guideline development. The guideline development group elaborated the final recommendations based on systematic review of scientific literature and critical evaluation of the evidence available using the grading of recommendations, assessment,

development, and evaluation (GRADE) approach. These guidelines are intended for health-care providers, HIV program managers, policy-makers, national treatment advisory boards, and other professionals involved in caring for people who either have or may be at risk of developing disseminated histoplasmosis.

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#### Authors contributions

All authors contributed equally in making this research successful.

#### Ethical approval

Ethical clearance (NBTL/TR6/ZA/182/vel3v) for the study was obtained from the Ethical Committees of National Tuberculosis and Leprosy Training and Referral Hospital Saye, Nigeria. Similarly, informed consent was obtained from each of the participants prior to recruitment into the study.

#### Competing interests

There was no any competing interest.

#### Funding

The research was not funded

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