Microbes and Infectious Diseases 2024; 5(2): 818-829

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Prevalence and susceptibility pattern of *Histoplasma capsulatum* among patients with pulmonary infection in Northern Nigeria according to sociodemographic, risk factors and clinical presentations

Rabiah Orchewa Osumah[1,2]*, Muhammad Hassan Isa Doko[2], Steven Olayeni Olonitola[2], Ocholi Yahaya[2], Husain Yahaya Ungokore[3] , Busayo Olalekan Olayinka[4]

1- University of Benin, Faculty of Life Sciences, Department of Science Laboratory Technology P.M.B. 1154, Edo State.
2- Ahmadu Bello University, Faculty of Life Sciences, Department of Microbiology, Zaria City – Nigeria.
3- Ahmadu Bello University, Faculty of Pharmaceutical Sciences, Department of Pharmaceutical Microbiology, Zaria City.
4- Usmanu Danfodiyo University, Faculty of Pharmaceutical Sciences, Department of pharmaceutical Microbiology, Sokoto State – Nigeria..

ARTICLE INFO

Article history:
Received 7 June 2023
Received in revised form 8 July 2023
Accepted 12 July 2023

Keywords:
Antifungal agent
E-test antifungal strip
Histoplasma capsulatum var duboisi
Histoplasmosis

ABSTRACT

Background: *Histoplasma capsulatum* is the causative agent of histoplasmosis, a dimorphic fungal disease. The study aimed at determining prevalence and susceptibility pattern of *Histoplasma capsulatum* among patients with pulmonary infection in Northern Nigeria. **Material and Methods:** This is a cross sectional and hospital-based study. Samples were collected from patients and questionnaires were administered. Three hundred and twenty-two sputum samples were collected and analysed with standard microbiological methods. The use of lactophenol cotton blue dye, revealed the presence of yeast like cells. Varieties differentiation was done by urease test. Sociodemographic and risk factors associated with histoplasmosis were determined by administering questionnaires to participants. Antifungal E-test strips were used to determine the susceptibility and data analysed by Statistical Package for Social Sciences version 20 at 5% significance level. **Results**: *Histoplasma var. duboisii* was isolated with 18(5.59 %) prevalence. Geriatric age (12.5 %), had the highest prevalence among the age groups in the study. Males (8.07 %), having high prevalence than females (3.10 %). The married and Civil servants had 6.25 %, and 8.69 % prevalence respectively and statistically associated with histoplasmosis. The immunocompromised and traders among the risk factors considered in the study, has an odd ratio of 1.026 and 1.060. Amphotericin B was most effective in this study. **Conclusion**: Histoplasmosis mimics tuberculosis in clinical presentations, hence, should be diagnosed alongside tuberculosis infection before commencement of therapy, awareness campaign, public policy should be made by the government on its presence and how to tackle the disease alongside AIDS and Tuberculosis.

Introduction

Histoplasmosis is a systemic fungal disease caused by *Histoplasma capsulatum* [1]. Human histoplasmosis is due to two varieties of the pathogen, *Histoplasma capsulatum* variety *capsulatum* and *Histoplasma capsulatum* variety *duboisii*, the former being the causative agent of classical histoplasmosis [2], while the latter is the etiologic cause for African histoplasmosis [3]. The fungus is primarily a saprophytic mold in soil (at a temperature of 25 ℃) containing rotten bird or bat

DOI: 10.21608/MID.2023.216101.1537
* *Corresponding author:* Osumah Orchewa Rabiah
E-mail address: *osuma.orchewa@uniben.edu.ng*

guano or enriched with organic nitrogen sources like animal excrements, or when grown in the laboratory as yeast form at less than 37 ºC [4]. It occurs by inhalation of the infectious spores produced by the dimorphic fungus *Histoplasma capsulatum* [5]. The environmental conditions present in areas of high endemicity are moderate climate with constant humidity [6] and infection occurs by the inhalation of microconidia by the host, deposition in alveoli and conversion to a parasitic yeast form in tissues. This germination and conversion can occur prior to or after ingestion by pulmonary macrophages [7].

The clinical manifestations of histoplasmosis range from asymptomatic infection to disseminated sepsis [8]. These manifestations depend mainly on the magnitude of exposure (i.e., the number of fungal particles inhaled), the immunological status of the host, and the virulence of the infective strain, indicating that environmental and genetic factors control the manifestations of the disease [9]. Additionally, in the setting of severe immunocompromised patients, such as individuals with AIDS, *H. capsulatum* strains previously not considered virulent are able to cause fatal disease [10]. In patients with immunodeficiency disorders, and especially in those infected with HIV, histoplasmosis is considered an opportunistic infection [11]. Additionally, in a high proportion of cases, this fungal infection is manifested as a severe disseminated process which often leads to death if it is not treated promptly. The true global burden of histoplasmosis is not well documented for the fact that there is limited data about the disease in Africa despite being endemic in some parts of the continent.

The treatment of histoplasmosis depends on the severity of the infection, clinical manifestation and individual risk factor. The drugs used to treat patients are amphotericin B, itraconazole, flucytosine, fluconazole, posaconazole, voriconazole, caspofungin [12].

Predisposing factors

Different conditions predispose an individual to disseminated histoplasmosis, of which are immunocompromised condition (example; acquired immune deficiency syndrome (AIDS)), administration of immunosuppressant therapy (these include, glucocorticoids, anti-rejection therapies in solid organ transplantation, or TNF-α inhibitor therapies, cancer patients), primary immunodeficiency, and people of geriatric age [13]

Explanation for the scientific background and the rationale

In Nigeria, Histoplasmosis is a burden that increased the death rate among Mycobacterial tuberculosis patients. This occurrence is a result of unawareness of the disease by clinicians and it mimicked tuberculosis in clinical presentations and hence is missed in diagnosis and treatment.

Specific Objective

The objective of this study is to determine the prevalence and susceptibility pattern of *Histoplasma capsulatum* in patients with pulmonary infection in Northern Nigeria according to sociodemographic, risk factors and clinical presentations.

Material and Methods

Study area

This study was conducted in Zaria, Nigeria. The ancient city of Zaria is a major city in Kaduna State, in Northern Nigeria as well as being a local government area, formally known as Zazzau [14]. Zaria is located between latitudes 11o04'00"N and 11o08' 800"N and longitude 7o36 ' 00"E and 7o44 ' 00"E [15]. The total area covered is about 2638.20 km^2 with an altitude of about 615 meters above sea level [16].

Study design

This was a cross sectional and hospital based research. Samples and questionnaires were collected and administered to patients under investigation.

Study population

This comprised patients with signs and symptoms of pulmonary infection attending National Tuberculosis and Leprosy Training Centre and Referral Hospital, Saye.

Inclusion criteria

Patients attending National Tuberculosis and Leprosy Training and Referral Hospital, Saye presenting with pulmonary diseases who gave consent were included in the study.

Exclusion criteria

Non-pulmonary disease patients presenting at National Tuberculosis and Leprosy Training and Referral Hospital Saye and those patients who did not give consent were excluded in the study.

Hypotheses

Ho = Pulmonary disease patients do not have histoplasmosis disease

Ha = 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 immunocompression, construction work, renovation of abandoned building, farming and gardening.

Collection of samples

Sputum samples were aseptically collected in a universal container from the pulmonary disease patients who attending National Tuberculosis and Leprosy Training Hospital Saye, Zaria, Nigeria by a qualified Medical Laboratory Personnel. A total of 322 sputa were collected from 322 pulmonary patients.

lsolation and identification of *Histoplasma capsulatum*

Preparation of sputum

Sputum samples were diluted with sterile distilled water and centrifuged at 2000 rpm to obtain a homogeneous solution prior to culturing [17].

Culturing of sputum samples

Sabouraud dextrose agar supplemented with cycloheximide + chloramphenicol agar (BD Difco, Sparks, USA) enriched with 10 % sheep blood was used to isolate the filamentous form (Mold) at a temperature of (25 ℃) for six weeks and brain heart infusion agar (BD Difco, Sparks, USA) enriched with 10 % sheep blood and supplemented with glutamine 5 %, penicillin 12 mg/ml and streptomycin 40 mg/ml was used to isolates yeast form (pathogenic) [18]. Petri dishes were incubated at 37 ℃ for 24 hours. Distinct colonies were inoculated into yeast extract agar slant for further use.

Microscopic examination

A pure yeast culture was placed on a clean glass slide for direct microscopic examination. A drop of lacto-phenol cotton blue was added to the fixed culture slide, allow to dry and visualize under 40X objective lens. The presence of yeast-like cells is considered a positive result for *Histoplasma capsulatum* [19].

Urease test

Urea agar was suspended in 900 mL of distilled water, boiled to dissolve completely and autoclaved at 121 ℃ for 15 minutes, agar was allowed to cool to 50 - 55 ℃ and aseptically added 100 ml of filtered sterile urea base to the cooled agar solution, mixed thoroughly and was distributed into sterile tubes and slants to solidify.

The surface of the solidified agar slant was streaked with overnight broth culture of *Histoplasma capsulatum* cells. The cap was leaved on loosely (to differentiate *Histoplasma capsulatum variety duboisii* from *Histoplasma capsulatum variety capsulatum*) and was incubated at 37 ℃ for 2 weeks. A positive result was considered when the color of the media changed from yellow to bright pink as a result of acid production [20].

Antifungal assay of the test drugs

Preparation of inocula

A 48 hours culture was used for the preparation of yeast suspension and the turbidity was adjusted to 0.5 McFarland Standard.

Determination of minimum inhibitory concentration

The E-test strips is a device used in this research, it consists of a predefined, continuous and exponential gradient of antibiotic concentration immobilized along a rectangular plastic test strip. After incubation, a drop shaped inhibition zone intersects the graded test strip at the inhibitory concentration (IC) of the antibiotic.

H. capsulatum pure culture was screened for susceptibility to antifungal agents (Minimum Inhibitory Concentrations test strips) (Liofilchem s.r.l Italy) by Agar diffusion method [20]. Mueller Hinton agar was prepared according to manufacturer's instructions and supplemented with 5 % glucose before dispensing into sterile petri dish and was allowed to solidify. A sterile cotton swab was dip into the standardized fungal inoculum, by rotating petri dish on a flat surface, the swab was used to streak the entire surfaces of the plates aseptically, inoculation was complete by running the swab around the rim of the agar. Plates were allowed to stand for 15 minutes for excess moisture to be absorbed before applying the test strips with a sterile forceps, and placed at the edge of the plate with the scale visible to the surface (facing upwards). Plates were incubated at 37 ℃ for 24 hours. Isolates were interpreted as susceptible, suboptimal MIC and resistant based on the mean plasma concentration as provided by Martindale [21].

Statistical procedures

Data was analysed 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** < 0.05 was taken to be statistically significant.

Results

Prevalence of *Histoplasma capsulatum* infection among the study participants was detected. Out of the 322 cultured sputa samples, 18 were positive for *Histoplasma capsulatum* giving a prevalence of 5.59 %

All of the 18 *H. capsulatum* positive sputum cultures out of the 322 examined isolates were urease negative showing that all samples were *Histoplasma capsulatum variety duboisii*.

Table 1 shows the result of prevalence of histoplasmosis among the study participants based on their sociodemographic factors naming; age, gender, marital status, and occupation. 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 not statistically significant.

According to gender, males had a higher prevalence of (8.07 %) than females (3.10 %). The difference was not statistically significant. Based on the marital status, those who were married recorded the highest prevalence (6.25 %), the difference was statistically significant (**p** = 0.00).

As for the factor of occupation, civil servants, recorded the highest prevalence (8.69 %), the difference was however statistically significant (**p** < 0.05).

As shown in **table (2)**, participants belonging to the group immunocompromised and trading among the risk factors considered in the study has an odd ratio of 1.026 and 1.060 showing that, they are associated with histoplasmosis.

The most common symptoms of Histoplasmosis experienced by patients in this study were headache, fever and chill **(table 3)**.

Antifungal susceptibility of the *Histoplasma capsulatum* isolates

Table 4 summarizes the in-vitro susceptibility of the 18 clinical isolates of *H. capsulatum* to three antifungal agents (flucytosine, itraconazole, and amphotericin B µg/ml). Among the 18 isolates 14(77.8 %) were susceptible to amphotericin B making it the most active agent with an MIC range of 0.023 – 0.125 µg/ml while 6(33.3 %) isolates were susceptible to itraconazole with an MIC of 2 µg/ml. For flucytosine, 10(55.6 %) isolates were susceptible while the remaining 8 isolates could not be classified as susceptible or otherwise, for the fact that the highest MIC on the test strip was suboptimal, which means that the antifungal test strip was not up to the peak plasma concentration required to inhibit *Histoplasma capsulatum*.

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

Age	**Number Examined**	**positive (%)**	**Df**	x^2	**P-value**
10-19	31	1(3.22)	7	11.33	0.13
20-29	74	4(5.40)			
30-39	72	6(8.33)			
40-49	48	3(6.25)			
50-59	43	1(2.32)			
60-69	25	1(4.00)			
70-79	21	1(4.76)			
80-above	8	1(12.5)			
	322	**18(5.59)**			
Gender					
Male	161	13(8.07)	1	2.88	0.09
Female	161	5(3.10)			
	322	**18(5.59)**			
Marital Status					
Married	224	14(6.25)	**2**	16.33	0.00
Single	70	3(4.28)			
Divorced	28	1(3.57)			
	322	**18(5.59)**			
Occupation					
Self employed	157	13 (8.28)	**3**	21.56	0.00
Unemployed	73	1(1.36)			
Civil Servant	23	2(8.69)			
Farming	69	2(2.89)			
	322	**18(5.59)**			

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

Variables	**Option**	**No Examined**	**No Positive (%)**	**Odd ratio**	**Lower limit**	**Upper limit**
Immune-com.	Yes No	1 321	0(0.00) 18(5.61)	1.026	1.008	1.044
Construction worker	Yes No	2 320	0(0.00) 18(5.63)	0.022	0.001	0.396
Farming	Yes	97	3(3.29)	0.866	0.801	0.939
	No	225	15(6.67)			
Traders	Yes	4	0(0.00)	1.060	1.032	1.089
	No	318	18(5.66)			
Others	Yes	218	15(6.88)	0.340	0.075	1.548
	No	104	3(2.88)			

Key: Others = No specific work or trade engaged by these group of people. Immuno-com= Immunocompromised individual

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
Fever	Yes	217	10(4.60)	1.028	1.006	1.052
	No	105	5(4.76)			
Chill	Yes	93	5(5.37)	1.163	1.071	1.262
	No	229	13(5.67)			
Headache	Yes	222	11(4.95)	1.201	1.011	1.307
	No	100	07(7.0)			
Chest discom.	Yes	310	16(5.16)	1.251	0.071	1.227
	No	12	02(16.67)			
Muscle ache	Yes	210	12(5.71)	1.341	0.256	7.028
	No	112	06(4.92)			
Sleeplessness	Yes	27	03(11.11)	0.772	0.098	6.107
	No	295	15(5.08)			

Key: Chest discom. = Chest discomfort

Table 4. Antifungals inhibitory pattern on *H. capsulatum* isolates

Isolates	Antifungal agents		
	*Peak Plasma Concentrations (µg/ml)		
	0.5 – 4(AMB)	2.0 (ITR)	50 – 70(FLU)
22	0.047 (S)	3.0 (R)	3.0 (S)
55	0.064 (S)	2.0 (S)	SMIC
61	0.047 (S)	3.0 (R)	0.75 (S)
188	0.047 (S)	2.0 (S)	SMIC
193	-	-	SMIC
210	-	-	SMIC
225	0.032 (S)	2.0 (S)	2.0 (S)
231	-	-	SMIC
263	-	-	SMIC
265	0.094 (S)	-	3.0 (S)
268	0.023 (S)	3.0 (R)	1.0 (S)
279	0.094 (S)	3.0 (R)	1.5 (S)
297	0.023 (S)	2.0 (S)	SMIC
300	0.047 (S)	12.0 (R)	SMIC
303	0.032 (S)	2.0 (S)	4.0 (S)
317	0.032 (S)	-	1.5 (S)
313	0.023 (S)	2.0 (S)	1.5 (S)
318	0.125 (S)	4.0 (R)	0.38 (S)

Key: SMIC = Suboptimal MIC (Not up to the mean plasma concentration in which the organism would have been inhibited), S = Susceptible, R = Resistant
- = No MIC,* = Martindale (2007),AMB = Amphotericin B (0.002 – 32 µg/ml),ITR = Itraconazole (0.002 -32µg/ml) ,FLU = Flucytosine (0.002 -32 µg/ml)

Figure1. Geographical position of the Nigeria showing the investigated area Kaduna State (according to Geographic Information System (GIS) Using Arc GIS 10.3 Software)

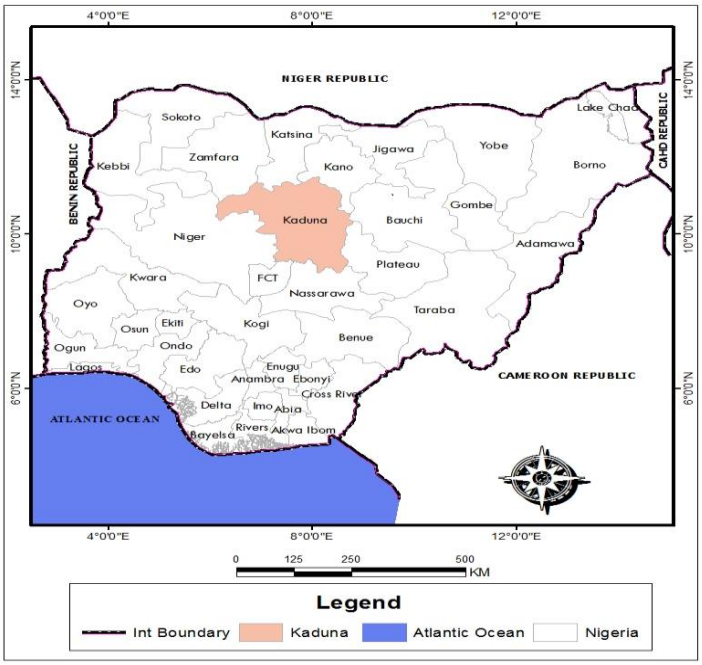


Figure 2. Kaduna State showing Zaira where the study area is located

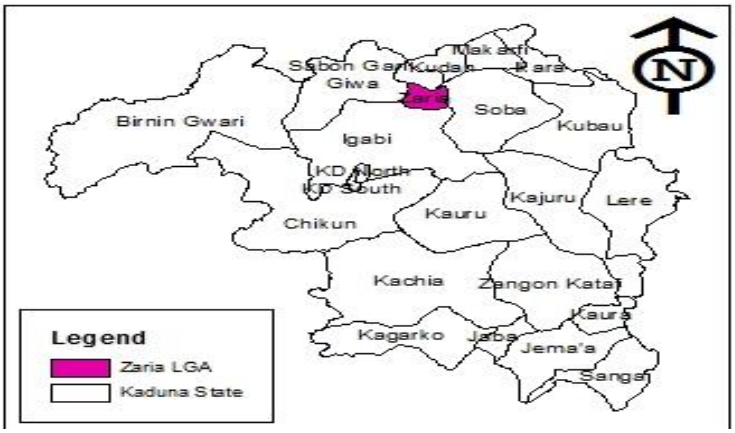
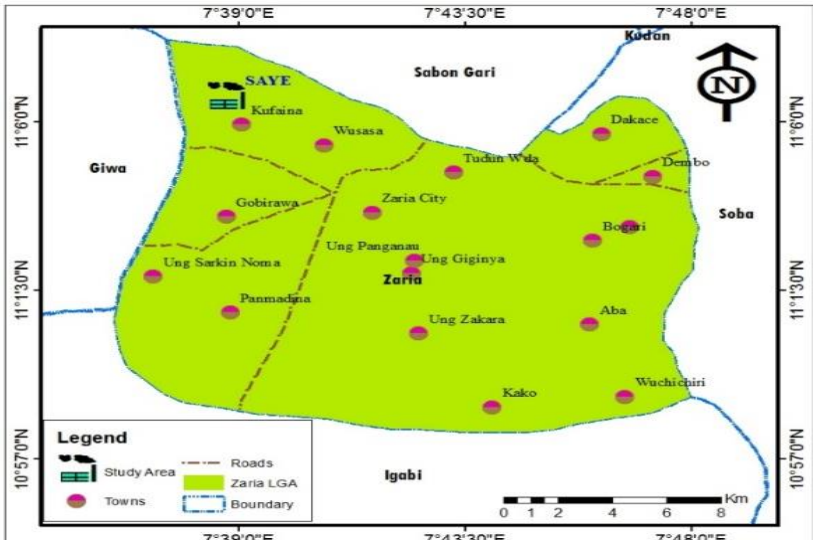


Figure 3. Map of Zaria showing Saye (according to Ministry of Land and Survey Kaduna State, 2000)



Discussion

Histoplasma capsulatum variety duboisii was the variety identified in this study, which is known to cause African histoplasmosis, although both varieties (*Histoplasma capasulatum variety capsulatum* and *variety duboisii*) of the fungus are present in Africa. This study therefore confirms the claim that it is associated with the continent of Africa, mostly in Central and West Africa [22]. This study confirms the claims its presence in Nigeria [23]. The prevalence of *H. capsulatum* was moderately low (5.56%) in the study population. This is about the first finding to be reported in this part of the country to the best of our knowledge, hence confirmed the presence of the infection in Northern Nigeria. The occurrence of histoplasmosis in the northern part of the country could be as a result of the favorable environmental condition prevailing, such as high humidity and temperature which have been reported to support the growth of *Histoplasma capsulatum* in a soil rich with higher nitrogen content and bats/bird's droppings [24]. Activities that disrupt soil during excavation, construction, demolition and renovation of buildings generate contaminated dust, hence, construction workers in endemic area are at a higher risk of contracting the disease [25].

The prevalence in this study is (5.59 %) which is low, as compared 6.5 % prevalence reported by **Gugnani et al.** [26] among the hospitalized patients, this difference may be due to the detection method used. Culture based method was used in this research, which is definitive and considered gold standard for the detection of histoplasmosis with 100 % specificity [27]. In the case of **Gugnani et al.** [26], Histoplasmin skin sensitivity test was used which is antibody based and prone to error of cross – reactivity as earlier reported in cases of sporotrichosis, aspergillosis, coccidioidomycosis, para coccidioidomycosis and blastomycosis [7, 6]. This cross-reactivity can lead to a false-positive result which may be responsible for the higher prevalence observed in the study by **Muotoe-Okafor et al.** [28].

The biochemical identification of histoplasmosis was based on urease test, which is considered a distinguishing factor between two varieties *capsulatum* and *duboisii* [20]. All the isolates were urease negative which shows that the isolates were all variety *duboisii* which is associated with the African Continent.

The prevalence of histoplasmosis in relation to age of the study participants was highest among the geriatric age group 80 – 89 years, although not statistically significant and significant for urine sample. 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.** [29] 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 [30].

The distribution of *H. capsulatum* infection across gender showed that males (8.1 %) had a higher prevalence than the female (3.1 %) though not statistically significant. Literature has it that the males are more susceptible to systemic infections, as reported in the liberation of which histoplasmosis is one, probably due to the fact that males engage more in activities that could predispose them to *Histoplasma* infection such as construction work, demolishing of building etc. [31].

H. capsulatum infection differed across the marital status of the participants with the married being more infected ($\mathbf{p} < 0.000$), 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 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 [32]

Across occupation of the study participants, the distribution showed that civil servants recorded the highest prevalence. Although they are civil servants, it is also possible that they engage in other activities that expose them to the infection of *Histoplasma* 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 *Histoplasma capasulatum* infection. A similar observation was documented by **Ekeng et al.** [33]and **Coffey et al.** [34] who reported that soil contaminated with birds / bats droppings is a factor for acquiring histoplasmosis.

Of the risk factors considered in the study, immunocompromisation with odd ratio (1.026) and trading with odd ratio (1.060) were factors associated with histoplasmosis, this may be due to immunodepression of the individual hence exposure

to opportunistic infection. This is equally observed by **Myint et al.** [35] who stated that immunologic factor and absence of antiretroviral treatment leads to acquisition of opportunistic infections. **Islam et al.** [36] 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 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,** [37] and **Avasthi et al.** [38] have reported that fomites contaminated with the conidia of *Histoplasma capsulatum* can cause any infection if exposed.

Fever, chill and headache with odd ratio (1.028), (1.163), (1.201) respectively are symptoms associated with histoplasmosis in the study, this agrees with the literature that headache, fever, chill is the most common symptom of histoplasmosis [38].

Of the eighteen isolates subjected to antifungal susceptibility test, fourteen (77.78 %) were susceptible to amphotericin B. This is in line with available literature that amphotericin B is the most effective drug for severe systemic mycoses [30] which was confirmed in this study, also, similar to the ones reported by **Delhom et al.** [39]. Amphotericin B binds the ergosterol of fungal cell membrane, creating pores where ions or cytoplasmic content leak out of the cell resulting in cell death. The means of administration of amphotericin B, intravenous, makes it less susceptible to abuse which can lead to resistance, as can be seen in other antifungals. Itraconazole and flucytosine were active, although the activity of flucytosine (0.002 – 32 µg/ml) could not be determined due to the suboptimal drug concentration on the E-test strip when compared to the peak plasma (50 -70 µg/ml) concentration

Summary

Prevalence of *Histoplasma* was 5.59 % and *Histoplasma var. duboisii* was the variety isolated from the study population. Geriatric and married people were found to be associated with *H. capsulatum duboisii* infection. Fever, chill and headache are symptoms statistically associated with histoplasmosis. Amphotericin B was the most effective antifungal drug against the isolates in this study.

Conclusions

An overall prevalence of 5.59% was obtained for histoplasmosis among the study participant, confirming the presence of the disease in this part of the country.

Histoplasma capsulatum var. duboisii was the identified strain in the study to be responsible for the histoplasmosis which is known to cause African type of histoplasmosis.

Demographic factors associated with histoplasmosis in the study included married and civil servants

Amphotericin B was found to be most active antifungal agent used in the study.

Acknowledgment

Our appreciation goes to Professor Olowosulu A.K and Professor Ella E.E for their fatherly advice during the course of carrying out this research and the management of National Tuberculosis and Leprosy Training Centre and Referral Hospital Saye, Kaduna – Nigeria for allowing us to collaborate with them. The research received no specific grant from any agency in the public, commercial, or not-for-profit sectors.

Funding

The research was funded by National Tuberculosis and Leprosy Training Centre and Referral Hospital Saye, Kaduna – Nigeria.

Conflict of interests

The authors declare that there is no conflict of interest related to this article

Authors Contributions

Rabiah Orchewa OSUMAH: The sole originator of the research investigation, data curation and Microbiological analysis, sample collections and writer of the original manuscript draft.

Muhammad. I. DOKO; Steven. O. OLONITOLA and Busayo. O. OLAYINKA: where project administrators, validated and supervisors the manuscript, and resources persons.

Ocholi YAHAYA and Husain.Y. UNGOKORE: They worked on data analysis and validation using Statistical Package for Social Sciences (SPSS) software and editing

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.

References

1- **Develoux, M., Amona, FM., Hennequin, C.** Histoplasmosis caused by *Histoplasma capasulatum variety duboisii*: A comprehensive review of cases from 1993 -2019. Clinical infectious diseases 2020;73(3) 1- 23

2- **Sahaza JH, Duarte-Escalante E, Canteros C, Rodriguez-Arellanes G, Reyes-Montes MR, Taylor ML.** Analysis of the genetic diversity and population structure of *Histoplasma capsulatum* clinical isolates from Mexico, Guatemala, Colombia and Argentina, using a randomly amplified polymorphic DNA-PCR assay. Epidemiology and Infection 2019; 147:e204. https://doi.org/10.1017/S0950268819000931

3- **Oladele RO, Olusole OA, Malcolm DR, David WD.** Histoplasmosis in Africa: An emerging or a neglected disease. PloS Journal Neglected Tropical Disease 2018a; Pp. 1-17.

4- **Mittal J, Ponce MG, Gendlina I, Nosanchuk JD.** *Histoplasma Capsulatum*: Mechanisms for Pathogenesis. Current Topical Microbiology and Immunology 2019; 422:157-191. doi:10.1007/82_2018_114.

5- **Deeper GS-Jr.** Outbreak of Histoplasmosis: The spores set sail. PloS Pathogen 2021; 14(9): e1007213

6- **Fayyaz J, Vydyula R, Walezyszyn MP, Klaus-Dieter L.** Histoplasmosis: Background, Pathophysiology, etiology. Journal of drugs and diseases 2020; 16(2):234 - 239

7- **Maiga AW, Deppen S, Scaffidi B, Baddley J.** Mapping *Histoplasma capsulatum* exposure, United State. Journal of Emerging Infectious Diseases 2018; 24(10):1835-1839

8- **Linder AK, Kauffman CA.** Histoplasmosis: Epidemiology, diagnosis and clinical manifestation. Current Fungal Infection Report 2019; 131:120-28

9- **Khalafa SA, Patela P, Carusob CR, Parrettb T, Brana A.** Central nervous system histoplasmosis as a gliosarcoma mimicker: The diagnostic dilemma of solitary brain lesions Case Report 2022; 27: e01354

10- **Mirza VU, Rodriguez VV.** Cave diving for a diagnosis: Disseminated histoplasmosis in the immunocompromised. Case Report 2018; 12:92-94

11- **Perez F, Caceres DH, Ford N, Ravasi G, Gomez BL, Pasqualotto AC, et al**. Summary of Guidelines for Managing Histoplasmosis among People Living with HIV. Journal of Fungi (Basel, Switzerland) 2021; 7(2):134. DOI: 10.3390/jof7020134. PMID: 33673384; PMCID: PMC7918769.

12- **Bongomin F, Kwizera R, Baruch JB, Asio LG, Otu A. A.** Treatment of Histoplasmosis [IntechOpen website]. 2020. Available at: http://dx.doi.org/10.5772/intechopen.92984

13- **Sayeed M, Benzamin MD, Nahar L, Rana M, Aishy AS.** Hepatic Histoplasmosis: An Update. Journal of Clinical and Translational Hepatology 2021; 1-4. DOI: 10.14218/JCTH.2020.00080

14- **Britannica. The Editors of Encyclopaedia**. Hausa [website] . Encyclopedia Britannica 2023 Available at: https://www.britannica.com/topic/Hausa.

15- **Adamu G, Usman AK, Sawa BA, Abdul Kareem B.** Analysis of the spatial distribution of global system of mobile communication base stations in Zaria Urban Area Zaria Kaduna state. Journal of Zaria Geographer 2016; 22(1):1-17.

16- **Ogunleye EK.** Utilization of remote sensing products in Kaduna State. A case study of Kaduna and Zaria metropolis unpublished PGD project. Department of Geography and Regional Planning. Ambrose Ali University Ekpoma, Edo State 2006; 10-15

17- **Sharp Laboratory Service. (2018)** Preparation of Laboratory Specimen. Pp 1-7

18- **Goughenour K.** *Histoplasma capsulatum*: Drugs and Sugars Graduate Program in Microbiology. The Ohio State University 2020; 45-90

19- **Wijayawardene NN, Kevin D, Hyde KD, Lumbsch HT, Jian-Liu JK, Phookamsak R.** Outline of Ascomycota. Fungal Diversity 2018.

20- **Buitrago MJ, Valero C.** Laboratory diagnosis of histoplasmosis: An update [IntechOpen website]. In Histoplasma and histoplasmosis 2020.Available at: https//doi.org/10.5772/ intechopen.93305

21- **Martindale.** The complete drug reference. 36th edition. Pharmaceutical press 2007; 523-551.

22- **Amona FM, Denning DW, Moukassa D, Develoux M, Hennequin C.** Histoplasmosis in the Republic of Congo dominated by African Histoplasmosis, *Histoplasma capasulatum variety duboisii*. PloS Neglected Tropical Diseases Journal 2021; 15(5): e0009318

23- **Oladele RO, Toriello C, Ogunsola FT, Ayanlowo OO, Foden P, Fayemiwo AS, et al.** Prior subclinical histoplasmosis revealed in Nigeria using Histoplasmin skin testing. PLoS ONE 2018b; 13(5): e0196224. https://doi.org/10.1371/journal.

24- **Oladele RO, Ayanlowo OO, Richardson MD, Denning DW.** Histoplasmosis in Africa: An emerging or a neglected disease? PloS Neglected Tropical Diseases 2018c; 12(1):e0006046.
DOI: 10.1371/journal.pntd.0006046

25- **De-Perio MA, Benedict K, Williams SL. Niemeier-Walsh C, Green BJ, Coffey C, et al**. Occupational Histoplasmosis: Epidemiology and Prevention Measures. Journal of Fungi 2012;7:510.
https://doi.org/10.3390/jof7070510

26- **Gugnani HC, Egere JU, Larsh H.** Skin sensitivity to capsulatum and duboisii Histoplasmin in Nigeria. Journal Tropical Medical Hygiene 1991; 94(1):24-6.

27- **Azar MM, Hage CA.** Clinical perspective in the diagnosis and management of Histoplasmosis. Clinic in Chest Medicine Journal 2017. (38-93):403-15

28- **Muotoe Okafor FA, Gugnani HC. Gugnani A.** Skin and serum reactivity among humans to Histoplasmin in the vicinity of a natural focus of *Histoplasma capsulatum var duboisii*. Mycopathologia 1996; 134:71-74. https://doi.org/10.1007/bf00436867

29- **Shojaei E, Walsh JC, Sangle N, Yan B, Silverman MS, Hosseini-Moghaddam SM.** Gastrointestinal Histoplasmosis Mimicking Crohn's Diseases. Open Forum Infectious Diseases 2021; Pp. 1 - 6

30- **Pakasa N, Biber A, Nsiangana S, Imposo D, Sumaili E, Muhindo H, et al.** African histoplasmosis in HIV-Negative Patients, Kimpese, Democratic Republic of the Congo. Emerging Infectious Diseases 2018; 24(11):2068-2070

31- **Evrard S, Caprasse P, Gavage P, Myriam V, Radermacher J, Hayette MP, et al.** Disseminated histoplasmosis: case report and review of the literature. Acta Clinica Belgica 2017; 73(4):1-8

32- **Ekeng BE, Edem K, Akintan P, Oladele RO.** Histoplasmosis in African children: clinical features, diagnosis and treatment. Therapeutic

Advances in Infectious Disease 2022; 9: 1–16 ttps://doi.org/10.1177/20499361211068592

33- **Coffery C., Park J., Toda, M.** Occupational Histoplasmosis: Epidemiology and Prevention measures. Journal of Fungi 2021; 1 – 14

34- **Myint T, Leedy N, Villacorta Cari E, Wheat LJ.** HIV-Associated Histoplasmosis: Current Perspectives. HIV AIDS (Auckl) 2020 19(12):113-125. doi: 10.2147/HIV.S185631. PMID: 32256121; PMCID: PMC7090190.

35- **Islam MS, Habib MR, Asha ET, Sharmin M, Ashraf M, Mahmud AA, et al.** Disseminated Histoplasmosis in Immunocompetent Patients Presented with Fever of Unknown Origin (FUO). Journal of Medicine 2023; 24(1):59-64. Available at: https://www.banglajol.info/index.php/JOM/article/view/

36- **Nega J, Taye S, Million Y, Rodrigo C, Eshetie S.** Antiretroviral treatment failure and associated factors among HIV patients on first-line antiretroviral treatment in Sekota. northeast Ethiopia. AIDS Research and Therapy 2020; 17:(39): 1-9 https://doi.org/10.1186/s12981-020-00294-z

37- **Avasthi D, Fatima H, Gill M, Avasthi S.** Disseminated Histoplasmosis in an Adult with Rheumatoid Arthritis Not on Biological Immune Modulators. Cureus 2021 17;13(6): e15709. doi: 10.7759/cureus.15709. PMID: 34277293; PMCID: PMC8285937.

38- **Nacher M, Adenis A, Blanchet D, Vantilcke V, Demar M, Basurko C, et al.** Risk factors for disseminated Histoplasmosis in a cohort of HIV-infected Patients in French Guiana. PloS Tropical Diseases 2014; 8(1): e2638

39- **Delhom R, Nelson A, Laux V, Wacklin-Knecht W, Heartlein M, Knecht W.** The antifungal mechanism of Amphotericin B elucidated in ergosterol and cholesterol containing membrane using neutrons. Journal of nanomaterial 2020; 10:2439.

Osumah R O, Doko M H I, Olonitola S O, Yahaya O, Ungokore H Y, Olayinka B O. Prevalence and susceptibility pattern of *Histoplasma capsulatum* among patients with pulmonary infection in Northern Nigeria according to sociodemographic, risk factors and clinical presentations. Microbes Infect Dis 2024; 5(2): 818-829.