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Detection of Opportunistic Fungal Pathogens in HIV/AIDS Patients Attending HIV Clinic, Federal Medical Centre Azare, Bauchi State Nigeria

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ABSTRACT

Opportunistic fungal infections are common in immunocompromised HIV patients, yet their impact in Bauchi, northeastern Nigeria, is understudied. The objective of this study was to detect and identify opportunistic fungal pathogens among HIV patients and to assess their risk factors. The study involved 385 HIV patients with opportunistic fungal infections symptoms, where urine, blood, and sputum samples were examined. Pathogens were isolated on potato dextrose agar, and standardized questionnaires were distributed to the patients to gather information on possible infection risk factors. Chi-square, univariate, and multiple logistic regression analyses examined relationships between the variables. The prevalence of opportunistic fungal infections among HIV patients was 25.71%. Out of the total positive urine samples, 38 (66.6%), 5 (8.7%), and 14 (24.5%) were *Candida spp Cryptococcus spp*, and *Aspergillus spp*, respectively. In blood samples, 42.9%, 16.7%, and 40.5% were positive for these fungi, but all sputum samples were negative. The presence of birds dropping and patients not on antiretroviral therapy (ART) were significantly associated with opportunistic fungal infections. Multivariate logistic regression analysis revealed that HIV patients residing in areas with birds dropping are 62 times more likely to develop opportunistic fungal infections compared to those without such exposure [AOR (95% CI): 61.965 (18.258–210.306)]. HIV patients not on antiretroviral therapy (ART) were at 98 times higher risk of developing opportunistic fungal infections than those on ART [AOR (95% CI): 98.475 (33.437–290.016)]. The study found *Candida spp Cryptococcus spp*, and *Aspergillus spp* in clinical samples, with *Candida spp* being the most common among immunocompromised individuals. *Aspergillus spp* and *Cryptococcus spp*, were less common. Furthermore, the study highlighted that the major infection risk factors were HIV patients not on antiretroviral therapy (ART), residing in areas with bird droppings and viral load.

Keywords: Opportunistic fungal infections (OFIs), bird droppings, ART, Viral load, *Candida* spp., *Aspergillus* spp., *Cryptococcus* spp.

INTRODUCTION

Many fungal infections are opportunistic, usually affecting people with weakened immune systems (Kaur *et al.,* 2016). The human immunodeficiency virus (HIV) weakens the immune system so that the body cannot resist the source of infection, leading to multiple infections in patients. The main cause of the morbidity and mortality of HIV-infected people is opportunistic infection, which is caused by a decrease in the patient's immunity due to a decrease in the CD4 count (Kaur et al., 2016). The modes of fungal infection vary worldwide depending on the type of fungus, host immune status, and site of infection (Kaushik *et al.,* 2015; Zhan *et al.,* 2015). Due to the increasing prevalence of

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immunocompromised populations in recent decades, invasive fungal infections have been considered a growing threat to human health (Miceli *et al.,* 2011). Among opportunistic fungal infections (OFIs), *C. albicans* and *A. fumigatus* infections account for most of the fungal infections in immunocompromised individuals, which are usually life-threatening (Pfaller *et al.,* 2007). Although *C. albicans* is the most common isolated organism, some studies have shown that non-*Albicans* species, including *Candida tropicalis, Candida krusei,* and *Candida glabrata,* are more common than *Candida albicans* (Jain *et al.,* 2014). The incidence of OFIs has increased, especially in hospitalized patients who are most at risk among HIV-infected patients (Magalhães *et al.,* 2015). Early specific diagnosis and follow-up treatment to combat these infections are not only concerns of Western hospitals but also crucial for developing countries, including Nigeria.

According to Kaur *et al.* (2016), the highest number of opportunistic infections is observed in patients aged 21-40, the most productive age group. It turns out that men are more often infected and are predominantly male in most age groups. The clinical manifestation of these infections may be acute, subacute, chronic, or episodic (Puebla, 2012). Involvement may be confined to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or gastrointestinal tract, or become systemic, such as sepsis, endocarditis, and meningitis (Puebla, 2012). Systemic opportunistic fungal infections (OFIs) are observed in patients with cell-mediated immune deficiency and those receiving aggressive cancer treatment, immunosuppression, or transplant therapy (Puebla, 2012). The management of serious and life-threatening opportunistic fungal infections remains severely hindered by delayed diagnosis and a lack of reliable diagnostic methods that allow the detection of fungemia and tissue invasion by opportunistic fungal infections (Puebla, 2012).

Opportunistic fungal infections (OFIs) are severe health problems among HIV-positive individuals worldwide (Kong *et al.,* 2007). Over several decades, the advent of the HIV epidemic and the increasing use of immunosuppressive drugs for severe medical conditions have dramatically increased the number of persons who are severely immunocompromised (Kaur *et al.,* 2016). Also, the range and the diversity of the fungi that cause infections have broadened. These led to a drastic increase in patients suffering from OFIs in African countries where health programs are seriously weak (Magalhães *et al.,* 2015). With the growing concerns about human immunodeficiency virus (HIV), there is a lack of information regarding the impact of opportunistic fungal infection in HIV-infected populations in northeastern Nigeria, particularly Bauchi state. The current study provided firsthand information on the type and nature of opportunistic fungal infections in HIV patients, their risk factors, and the relationship between the immune status of the patients and the number and kind of infections to ensure positive patient outcomes and reduced morbidity and mortality rate of the patients.

MATERIALS AND METHODS

Study Setting, Design, and Ethical Approval

The study was conducted at the HIV clinic at Federal Medical Center Azare Bauchi State. It is one of the standard tertiary medical centers owned by the Federal Government of Nigeria, providing all kinds of medications and treatments. The centre is located at No. 5 Sule Katagum Road, Azare Bauchi State, Nigeria. Azare is the headquarters of Katagum local government, Bauchi State of Nigeria.

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This study is a component of cross-sectional research conducted following the acquisition of written consent for sampling and ethical approval from the ethical committee of Federal Medical Center Azare Bauchi State bearing reference number FMCA/COM/36. The data were randomly collected from the HIV patients enrolled in the HIV Clinic Federal Medical Center Azare at a single point in time.

The research was approved for the stated patients from the Research and Ethics Committee, Federal Medical Center Azare, Bauchi State, Nigeria. The study participants were informed about the research, and their participation in the work was voluntary.

Study Population and Sample Size determination

The study population includes HIV patients of all ages and sexes attending the HIV Clinic, Federal Medical Center Azare, with signs and symptoms of fungal infections, but all HIV patients with no signs and symptoms of the infections were excluded. The sample size was determined based on the assumption that the study area did not possess any available prevalence estimates for OFIs among HIV patients. Thus, to get a minimum sample size, a 50% prevalence rate for opportunistic fungi pathogens among HIV patients was used as an estimate.

$$N = \frac{Z^2 pq}{d^2}$$

A minimum sample size of 385 was determined, utilizing a 95% Confidence level and precision of 5%. The study ultimately incorporated data from 385 individuals diagnosed with HIV. All participants were informed of the study's purpose, and 385 study participants provided informed written consent to engage in the research.

Isolation of the Pathogens

The pathogens were isolated on Potato dextrose agar and Sabouraud's dextrose agar, where the plates containing the prepared media were inoculated with the test specimen and incubated at 35^{o}C with periodic checking for growth (Naveena and Joy, 2014).

Identification of the fungal isolates

Fungal isolates were mophologically identified as described below by Naveena and Joy (2014).

Gram staining

A smear of the organism to be identified was prepared and heated gently to get it fixed. The slide was flooded with 0.5 % crystal violet and left for 30 sec. Then rinse gently with water. The slide was then flooded with Lugol's iodine and allowed to remain for 30 seconds and wash off with water. This was followed by decolorization with 95% ethanol and rinse with water. The slide was then flooded with 0.1 % counterstain safranin and allowed to stand for about 1min. Finally, the slide was washed with water and blotted dry. The slide was examined using an oil immersion objective for cell morphology and Gram reaction.

Lactophenol Cotton Blue (LCB)

One drop of the lactophenol cotton blue stain was placed on a clean slide. A small portion of the test organism was picked up and mixed with the stain. A cover slip was gently pressed on the slide to avoid air bubbles and to make a thin mount. The prepared slide was examined under low power (x10) with reduced lighting and then switched to high power (x40) to check for suspected fungal structures.

Indian Ink

A drop of Indian ink was placed onto a clean glass slide. A small quantity of the specimen

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was added to the slide surface beside the ink before mixing it into the ink. A cover slip was placed over the smear to avoid air bubbles. The slide was then examined with a high-power lens.

Chromagar Candida

Ten grams (10g) of Chromopeptone, 20.0g of Glucose, 2.0g of Chromogen Mix, and 15.0g of Agar were dissolved in 1000 ml of distilled water. The mixture was autoclaved at 121°C for 15 minutes and cooled before pouring into a plate. The plate was inoculated with the test organism, incubated for 20 to 48 hours at 35° C, and observed for the appearance of different colours.

Corn Meal Agar

Seventeen grams (17g) of Corn Meal Agar (CMA) was Suspended in 1000ml of distilled water and boiled to dissolve the medium completely. Exactly 7ml of Tween 80 was added and autoclaved at 121°C for 15 minutes. This was allowed to cool, poured into plates, and left for solidification. Three consecutive parallel scratches were made on the surface of the Corn Meal Agar plates and the bottom. Using an inoculating loop, a streak was made across the three scratches. A coverslip was flamed and placed over the center of the inoculation scratches. A positive control of a known chlamydospore-producing isolate was prepared. The plates were incubated at 25°C and examined microscopically at 24, 48, and 72 hours for the production of chlamydospores under a low power objective.

Germ Tube Test

The germ tube medium (human serum) was inoculated using a needle with a yeast sample and Incubated at 37ºC for 2 to 4 hours. After incubation, a drop of serum was withdrawn from the test tube and placed onto a glass slide. The slide was covered with a cover slip and examined for the presence of germ tubes.

Cryptococcus Antigen Detection Test

Twenty-five microliters (25 µl) of the Cryptococcus Antigen Positive Control, Negative Control, and the specimen were placed onto separate rings on a ring slide. Then, 25 microliters (25 µl) of Cryptococcal Latex were added to each ring. Using separate applicator sticks, the contents of each ring were thoroughly mixed. The slide was then gently rocked for 5 minutes at room temperature. The reactions were observed and recorded immediately afterward.

Statistical Analysis

Data analysis was carried out using the SPSS version 27.0 software package. A chi-square test, univariate and multivariate logistic regression were employed to explore potential relationships between the independent variables and the outcome variable. The association between the independent variables and the outcome was assessed by calculating crude odds ratios and 95% confidence intervals to measure the strength of the relationships

RESULTS

Sociodemographic Characteristics

The study also revealed that, out of the 385 participants enrolled, 271 (70.4%) were females and 114 (29.6%) were males. Of all the participants enrolled in the current study, 24 (6.2%) patients were below 20 years, 94 (24.4%) aged between 20-30 years, 112 (29.1%) aged between 31-40 years, 100 (26.0%) aged between 41-50 years while 55 (14.3%) were above 50 years. Moreover, 37 (9.6%) of the patients were single, 283 (73.5%) were married, and the remaining 65 (16.9) were separated. Likewise, 169 (43.9%) of the patients were housewives, 45 (11.7%) were civil servants, 31 (8.1%) were farmers, 59 (15.3%) were businessmen and women, 22 (5.7%) were students while 59 (15.3%) were

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in other categories. Furthermore, 34 (8.8%) of the patients were living in cities, 237 (61.6%) were from towns, and 114 (29.6%) were from villages (Table 1).

Table 1: Sociodemographic factors associated with Opportunistic fungal infections (OFIs)in HIV patients.

Variables	Category	Number of respondents	Percentage (%)
Gender	Female	271	70.4
	Male	114	29.6
Age	<20	24	6.2
	21-30	94	24.4
	31-40	112	29.1
	41-50	100	26.0
	>50	55	14.3
Marital status	Single	37	9.6
	Married	283	73.5
	Separated	65	16.9
Occupation	civil servant	45	11.7
	Farmer	31	8.1
	Business	59	15.3
	Housewife	169	43.9
	Student	22	5.7
	Others	59	15.3
Locality	City	34	8.8
	Town	237	61.6
	Village	114	29.6

Prevalence of opportunistic fungal infections (OFIs) among HIV patients

A total of 385 participants with HIV/AIDS who met the eligibility criteria were included in the study. Ninety-nine patients had OFIs, which indicated a prevalence of 25.71%. The organisms isolated were *Candida spp, Cryptococcus spp,* and *Aspergillus spp*. Out of the total positive samples collected from the urine, 38 (66.6%), 5 (8.7%), and 14 (24.5%) were *Candida spp, Cryptococcus spp,* and *Aspergillus spp,* respectively. In addition, 18 (42.9%), 7 (16.7%), and 17 (40.5%) of the blood samples were tested *Candida spp, Cryptococcus spp,* and *Aspergillus spp* positive, respectively. However, all the sputum samples collected tested negative, as shown in Table 2.

Table 2: Distribution of opportunistic fungi isolated from the clinical samples.

Isolates	Urine	Blood	Sputum
Candida spp	38 (66.6%)	18 (42.9%)	0
Cryptococcus spp	5 (8.7%)	7 (16.7)	0
Aspergillus spp	14 (24.5%)	17 (40.5%)	0
Total	57	42	0

OFIs have been identified in HIV patients receiving care at FMC Azare, Bauchi State. Among the 385 HIV patients participating in this study, 99 (25.7%) were infected with OFIs. The findings indicate that males exhibited a higher prevalence (30.7%) compared to females (30.3%); nevertheless, the difference was not statistically significant ($\chi2 = 0.007$: df = 1, p = 0.931). Participants aged 31-40 exhibited the highest frequency at 31.3%, whereas individuals under 20 demonstrated the lowest incidence at 29.2%. Statistical analysis indicated no correlation between the prevalence of infection and age group ($\chi2 = 0.086$: df = 4, p=0.999). HIV patients with a marital status of separation exhibit the highest prevalence at 30.8%, in contrast to those who are married at 30.4% and single at 29.7%. The statistical analysis indicates no significant difference ($\chi2 = 0.012$, df = 2, p = 0.994). Human Immunodeficiency Virus Students exhibited the highest prevalence of OFI infection at 36.4%, followed by farmers at 32.3%. Civil servants exhibited the lowest percentage at 26.7%, although the difference was not statistically significant ($\chi2 = 0.722$, df = 5, p = 0.982). Regarding locality, village inhabitants have the highest frequency at 30.7%, compared to 30.4% for those residing in towns and 29.4% for city dwellers. The results suggest no statistically significant difference ($\chi2 = 0.722$, df = 5, p = 0.982), as presented in Table 3.

Table 3: Chi square test of the Sociodemographic characteristics associated with Opportunistic fungal infections (OFIs) in HIV patients.

Variables	Frequency (%)	Positive N (%)	χ^2	p-value
Sex				
Male	114	35 (30.7)	0.007	0.931
Female	271	82 (30.3)		
Age				
<20	24	7 (29.2)	0.086	0.999
21-30	94	28 (29.8)		
31-40	112	35 (31.3)		
41-50	100	30 (30.0)		
>50	55	17 (30.9)		
Marital Status				
Single	37	11 (29.7)		
Married	283	86 (30.4)	0.012	0.994
Separated	65	20 (30.8)		
Occupation				
civil servant	45	12 (26.7)	0.722	0.982
Farmer	31	10 (32.3)		
Business	59	18 (30.5)		
Housewife	169	51 (30.2)		
Student	22	8 (36.4)		
Others	59	18 (30.5)		
Locality				
City	34	10 (29.4)	0.021	0.990
Town	237	72 (30.4)		
Village	114	35 (30.7)		

The study demonstrated that HIV patients living in areas with bird droppings have the highest prevalence of 88.5%, in contrast to a rate of 13.4% among those living in areas

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without such exposure. The difference was statistically significant (χ2 = 179.456, df = 1, p = 0.001). Differences in other risk factors for OFIs are evident, including dust and moisture, patients visiting caves, working in dusty areas, residents in moist and warm places, and the use of covered footwear. However, none of these factors exhibited statistically significant differences (p>0.05). Study participants not receiving antiretroviral therapy (ART) showed a higher prevalence of OFIs compared to those undergoing ART, with a statistically significant difference (χ2 = 250.577, df = 1, p = 0.001), as illustrated in Table 4.

Table 4: Chi square test of the risk-associated factors of opportunistic fungal infections (OFIs) in HIV Patients.

Variables	Frequency (%)	Positive N (%)	χ^2	p-value
Presence of bird droppings				
No	298	40 (13.4)	179.456	0.001
Yes	87	77 (88.5)		
Presence of dust and moisture				
No	275	89 (32.4)	1.773	0.183
Yes	110	28 (25.5)		
Visiting caves				
No	338	102 (30.2)	0.059	0.808
Yes	47	15 (31.9)		
Working in dusty areas				
No	161	49 (30.4)	0.001	0.987
Yes	224	68 (30.4)		
Staying at moist and warm				
No	369	112 (30.4)	0.006	0.939
Yes	16	5 (31.3)		
Using cover shoes				
No	380	115 (30.3)	0.221	0.638
Yes	5	2 (40.0)		
ART				
No	134	106 (79.1)	230.577	0.001
Yes	251	11 (4.4)		

Univariate and multivariate logistic regression analyses of the risk factors associated with OFI among HIV patients

We performed univariate and multivariate logistic regression analyses to assess the independent association between the risk factors OFI associated with HIV infection. The univariate analysis demonstrated that the risk factors associated with OFI among the HIV infection among the study participant were significantly associated with the presence of birds dropping in the environment [COR (95% CI): 49.66 (23.738-103.909)] and use of ART [COR (95% CI): 82.597 (39.648-172.073)]. The multivariate model was fitted, and both presence of birds dropping in the environment [AOR (95% CI): 61.965 (18.258-210.306)] and the use of ART [AOR (95% CI): 98.475 (33.437-290.016)] were significantly associated with OFIs (Table 5).

The study also indicated that 268 patients with a mean viral load of 270.67 ± 89.13 copies/ml tested negative, whereas 19 patients with a mean viral load of 1096.31 ± 15.17 copies/ml exhibited one form of opportunistic infection, and 31 patients had a mean viral load of 2922.29 ± 388.62 copies/ml exhibited two distinct infections, while sixty-seven copies/ml, with a mean viral load of 4559.63 ± 312.18 copies/ml, presented three different infections.

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No patient was identified with more than three distinct infections (Table 6).

Table 5: Univariate and multivariate logistic regression of the risk factors associated with OFIs in HIV Patients at FMC, Azare Northeast Nigeria.

Variables	Total	OFI Pos. N (%)	COR (95% CI)	p-value	AOR (95% CI)	p-value
Presence of bird droppings						
No	298	40 (13.4)	1			
Yes	87	77 (88.5)	49.66 (23.738-103.909)	0.001	61.965 (18.258-210.306)	0.001
Presence of dust and moisture						
No	275	89 (32.4)	1			
Yes	110	28 (25.5)	0.714 (0.434-1.174)	0.184		
Visiting caves						
No	338	102 (30.2)	1			
Yes	47	15 (31.9)	1.085 (0.563-2.090)	0.808		
Working in dusty areas						
No	161	49 (30.4)				
Yes	224	68 (30.4)	0.996 (0.642-1.547)	0.987		
Staying at moist and warm						
No	369	112 (30.4)				
Yes	16	5 (31.3)	1.043 (0.354-3.072)	0.939		
Using cover shoes						
No	380	115 (30.3)	1			
Yes	5	2 (40.0)	1.536 (0.253-9.317)	0.641		
ART						
No	134	106 (79.1)	1			
Yes	251	11 (4.4)	82.597 (39.648-172.073)	0.001	98.475 (33.437-290.016)	0.001

Table 6: Relationship between mean viral loads of the patients and the number of infections.

Number of infections	N	mean viral load (copies/ml)
Negative	268	270.67 ± 89.13
1	19	1096.31 ± 15.17
2	31	2922.29 ± 388.62
3	67	4559.63 ± 312.18
above 3	0	-
Total	117	

Key: ±: standard deviation of the data, N: number of patients

DISCUSSION

While HIV is the etiological agent of AIDS, the primary contributors to morbidity and mortality in these patients are opportunistic infections due to diminished humoral and cell-mediated immunity (Jain *et al.,* 2014). Opportunistic infections encompass viral, parasitic, bacterial, and fungal types. Fungal

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agents are universally present and are the predominant source of life-threatening infections in immunocompromised patients. Our study highlighted the prevalence and associated risk factors of OFIs among HIV patients in the HIV Clinic, Federal Medical Center Azare, Bauchi State, Nigeria.

The 25.7% observed in this study closely corresponds with the 29.0% prevalence rate reported in India (Kaur, *et al.,* 2016). The prevalence recorded in this study is lower than that reported in several regions of Nigeria, including Borno (Bukar *et al.,* 2017) and Ebonyi (Okorie *et al.,* 2018), which had prevalence of 53.67% and 47.50%, respectively. Similar trends of higher prevalence rates of 47.45% in India (Sharma *et al.,* 2023) and 51.66% in Nepal (Sah *et al.,* 2018) have been recorded in other regions.

In our study, *Candida spp* constituted 66.6%, followed by *Aspergillus spp* at 24.5% and *Cryptococcus spp* at 8.7%. Our result is similar to the study reported by Okorie *et al.* (2018) in Ebonyi, southeastern Nigeria, which found that *C. albicans w*as the most predominant species isolated from HIV patients, accounting for 42.1% of the fungal isolates. This finding aligned with the report of Kaur *et al.* (2016) in India, who identified a high prevalence of *C. albicans* (86.5%) among patients with OFIs. The results are also similar to the studies conducted in other parts of the world, such as Nepal (Sah *et al.,* 2018). Likewise, earlier research by Jain *et al.* (2014) and Pfaller et al. (2007) identified *Candida esophagitis* as a predominant opportunistic infection in HIV patients.

In urine samples, *Candida. spp* (66.6%) predominates, followed by *Aspergilus spp* (24.5%); the relative proportions seen in this study align with those reported by Kaur *et al.* (2016) in India, but the absolute values differ significantly. Similarly, previous studies by Jain *et al.,* (2014) and Pfaller *et al.* (2007) also documented one of the *Candida* species as a leading opportunistic infection among HIV patients. The predominance of *Candida* spp and the identification of *Aspergilus spp* in urine samples from HIV/AIDS patients may indicate their distinct pathogenic mechanisms and host susceptibilities. *C. albicans*, a commensal organism of mucosal surfaces, flourishes in immunocompromised individuals by exploiting diminished cellular immunity, especially when CD4+ T-cell counts fall below 200 cells/μL, hence impairing Th17-mediated mucosal defenses (Pfaller and Diekema, 2007). Colonization or infection of the urinary tract by *C. albicans* is exacerbated by indwelling catheters, diabetes, or previous antibiotic administration, which disrupts natural flora (Sobel *et al.,* 2011).

Conversely, the identification of *Aspergilus spp* in urine is rare but may signify disseminated infection may be linked to environmental exposure as haematogenous dissemination from original pulmonary sites can infect the kidneys in advanced AIDS when the CD4 count is less than 50 (<50 cells/μL) (Denning *et al.,* 1991). These findings underscore the necessity for regular fungal screening and prompt antifungal treatment in HIV/AIDS patients to reduce the risks of systemic opportunistic infections.

In blood samples, *Candida spp* is the most frequently isolated pathogen (42.9%), followed by *Aspergilus spp* (24.5%), while *Cryptococcus spp* are the least isolated (16.7%). The predominance of *Candida spp* and *Aspergilus spp* in blood samples, with *Cryptococcus spp* being the least frequently isolated, presumably indicates variations in pathogenesis, host immunological status, and diagnostic difficulties (Pfaller and Diekema, 2007). The low incidence of *Cryptococcus spp* in the blood may indicate its affinity for the

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central nervous system (CNS) and lungs, with cryptococcemia generally manifesting transiently during widespread illness. Blood cultures exhibit lower sensitivity for cryptococcosis than cerebrospinal fluid (CSF) antigen testing, and the preventive administration of fluconazole in HIV clinics may inhibit cryptococcal proliferation (Jarvis *et al.,* 2012).

The absence of significant statistical differences in the prevalence of opportunistic fungal pathogens across diverse sociodemographic characteristics, including gender, age, marital status, occupation, and locality, indicates that these infections are predominantly determined by the level of immunosuppression rather than demographic variables. For example, *Candida* and *Aspergillus* infections are opportunistic phenomena that manifest uniformly in populations when cell-mediated immunity is severely compromised, irrespective of age, gender, or occupation (Antinori et al., 2018). This discovery corresponds with the comprehension that HIV/AIDS-associated fungal infections are opportunistic, emerging from weakened immune defenses rather than external sociodemographic factors (Kaplan *et al.,* 2009).

The results from the present study revealed a significant association between the exposure to birds dropping in the environment and OFIs in HIV/AIDS patients. HIV patients residing in areas with bird droppings were 62 times more likely to develop OFIs compared to those without such exposure [AOR (95% CI): 61.965 (18.258-210.306)], emphasizing the role of environmental factors. This agrees with previous studies by Nweze et al. (2015) in Nigeria and Abulreesh *et al.,* (2015) in Saudi Arabia, indicating that exposure to bird droppings significantly increases the risk of fungal infections, particularly *Cryptococcus neoformans* infections. The association can be elucidated by bird droppings, especially from pigeons, a reservoir for harmful fungi like *C. neoformans* (Ghaderi *et al.,* 2019). Birds dropping, such as pigeon droppings, have been reported as the major environmental source of *C. neoformans* and many other pathogens in immunocompromised individuals in several countries (Abulreesh *et al.,* 2015). These fungus flourish in the nutrient-dense environment of bird droppings and can become airborne, heightening the risk of inhalation in immunocompromised individuals (Ghaderi *et al.,* 2019). The high adjusted odds ratio (AOR = 61.97) observed in the present study for bird droppings suggests that individuals exposed to environmental contamination are at remarkably higher risk of cryptococcosis infection.

Additionally, the study demonstrated a statistically significant correlation between ART use and reduced OFI prevalence. Patients not on ART were 98 times more likely to develop OFIs than those on ART [AOR (95% CI): 98.475 (33.437-290.016)]. This reinforces the findings of Patel et al. (2012) and Okoye and Picker (2013), which highlighted the effectiveness of ART in mitigating opportunistic infections among HIV patients. This may also be ascribed to immune reconstitution inflammatory syndrome (IRIS), a disorder wherein restoring immunological function via ART provokes an excessive inflammatory response to latent or undiscovered infections, including fungal pathogens (Nweze *et al.,* 2015).

The relationship between viral load and OFIs was also explored. Patients with higher viral loads exhibited an increased number of infections. For instance, individuals with a mean viral load of 4559.63 ± 312.18 copies/ml presented three distinct infections. This finding is supported by Laurie (2017), who

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posited that higher viral loads correlate with lower CD4 counts, predisposing patients to OFIs. The study also aligned with Kaur *et al.,* (2016), who observed increased OFI prevalence among patients with CD4 counts below 500. The findings suggest that targeted public health strategies, including environmental decontamination, enhanced ART coverage, and routine monitoring of viral load and CD4 counts, could significantly reduce OFI prevalence among HIV patients. Furthermore, the role of occupational and environmental factors warranted attention for comprehensive OFI management strategies.

CONCLUSION

The study conducted a comprehensive analysis and found that Candida. spp., a type of opportunistic fungal infection (OFI), is the most frequently identified pathogen among HIV patients across various clinical samples. Key risk factors contributing to the incidence of these infections include limited access to antiretroviral therapy (ART) for HIV patients, living in environments where bird droppings are common, and having a high viral load. Notably, the research did not pinpoint any sociodemographic factors linked to the risk of these infections. The primary aim of this study is to enhance the knowledge of healthcare providers, empowering them to make accurate diagnoses and deliver timely treatment for these infections, particularly in resource-limited areas of Nigeria where medical resources may be scarce.

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