Isolation and evaluation of *Candida* species and their association with CD4$^+$ T cells counts in HIV patients with diarrhoea.

Ayobami Awoyeni[1], Olarinde Olaniran[1], Babatunde Odetoyin[1], Rachel Hassan-Olajokun[1], Bolatito Olopade[1], David Afolayan[2], Oluwakayode Adekunle[1]

1. Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Science, College of Health Sciences. Obafemi Awolowo University, Ile-Ife, Osun-State.
2. Multidisciplinary Laboratories, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun State.

Abstract

Background: Gastrointestinal infection is one of the most common infections among HIV patients. *Candida* spp have been implicated in the aetiology of chronic diarrhoea in HIV patients, but little is known about this in Nigeria.

Objectives: We determined the prevalence of faecal candidiasis in HIV patients in relation to diarrhoea, CD4 counts, and other socio-demographic factors and the spectrum of *Candida* isolates involved.

Methods: One hundred and fifty four HIV patients were investigated. *Candida* species were identified by standard techniques. Socio-demographic and clinical information was obtained from the patients using a structured questionnaire. The CD4 count was estimated using a single platform flow cytometer.

Results: *Candida* overgrowth was detected in 61 (39.5%) HIV patients, and diarrhoea was associated with candidiasis in the subjects (P=0.001). Candidiasis was commonly detected among subjects in the 29-39 years' age group. A CD4 count below 200 cells/mm^2 (62.3%) was a risk factor for acquiring candidiasis among HIV patients (P=0.001). *Candida albicans* (65.6%) was the most frequently recovered species followed by *Candida krusei* (16.4%) and *Candida tropicalis* (14.8%).

Conclusion: Candidiasis is an important opportunistic infection in HIV-patients in Ile-Ife. There is need for regular checks for opportunistic infections, including *c*andidiasis in HIV patients to monitor disease progression and prevent subsequent complications.

Keywords: *Candida* species, CD4$^+$ T cells counts, HIV, diarrhoea.

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Introduction

The acquired immune deficiency syndrome caused by the human immunodeficiency virus (HIV) is the most important public health problem of the 20th century.[1,2] According to 2014 UNAIDS reports, only 9% of all people living with HIV globally live in Nigeria.[3] Even though the prevalence of HIV among adults is remarkably small (3.2%) in Nigeria compared with other sub-Sahara African countries like South Africa (19%) and Zambia (12.5%), the size of Nigeria's population means that there were 3.2 million people living with HIV in 2013.[3]

Nigeria, together with South Africa and Uganda account for almost half of all annual new HIV infections in sub-Saharan Africa. This is despite achieving a 35% reduction in new infections between 2005 and 2013.[3,4]

Despite the widespread HIV awareness programme going on at present, many patients either go undiagnosed or present late with multiple infections.[5] In infected individuals, the emergence of opportunistic infections is due to the unique pathogenesis of the virus which decreases the CD4 cells.[6] Opportunistic infections are a major cause of morbidity and mortality in such patients. Infectious microbial agents causing opportunistic infections could be asymptomatic or symptomatic in immune competent in-

Corresponding author:
Babatunde Odetoyin,
Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Science, College of Health Sciences.
Obafemi Awolowo University, Ile-Ife, Osun-State.
Mobile Phone: 08036968027
E-mail: odetoyin@yahoo.com

dividuals and often self-limiting. Nevertheless, in immune suppressed individuals and individuals with malignancy, these factors lead to a severe life-threatening disease.[7]

Candida is the most frequently encountered fungal infection of the GIT.[8] *Candida* species are often isolated from the stool samples of patients with diarrhoea, especially those living with AIDS. Gastrointestinal infections are commonly seen among HIV patients. Diarrhoea is a common clinical manifestation of these infections.[9] HIV-related diarrhoea is multi-factorial. The etiologic agents of diarrhoea include bacteria, parasites, fungi, and viruses.[10] *Candida* spp. have been implicated in the aetiology of chronic diarrhoea in HIV patients.[11]

Candida infection in humans is normally controlled by the immune system.[12] This implies that immunocompromised state such as HIV/AIDS render the host susceptible to a wide range of infections including fungal infections.[12] For instance, a high incidence of candidiasis has been shown in individuals with limited neutrophil functions and people with immune compromised conditions like HIV/AIDS.[13] On the other hand, HIV negative individuals may experience candidiasis when the immune system is temporarily depressed by other factors like malnourishment, chemotherapeutic agents, and widespread use of antibiotics.[14] A distinct increase has been noted in the proportion of cases resulting in infection with non-*albican Candida* species.[13]

A variety of *Candida* species are responsible for causing opportunistic fungal infections. However, *C. albican* is the most frequent etiologic agent followed by *C. tropicalis, C. parapsilopsis* and *C. glabrata*.[13] *C. albican* is part of the normal endogenous floral and its infections are believed to be endogenous in origin.[8]

In Nigeria, there are reports of isolation of *Candida* species in oral infections , but little is known about faecal candidiasis in relation to diarrhoea. Therefore, this study was conceived to determine the prevalence of faecal candidiasis in HIV patients, the relationship between faecal candidiasis and diarrhoea, CD4 count and other socio-demographic factors, and the spectrum of *Candida* isolates involved infaecal candidiasis.

Materials and methods

Location of study

This study was conducted between July 2013 and September 2013 on HIV/AIDS patients recruited at Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria. All patients agreed to participate in the study by signing an informed consent form approved by the Ethics and Research Committee of the hospital.

Subject selection

One hundred and fifty-four HIV/AIDS patients were included in this study, out of which 100 were patients with diarrhoea. Relevant information, such as age, gender, marital status, status of antiretroviral therapy, onset and duration of diarrhoea, sources of drinking water, toilet facilities, and occupation was obtained from the patients using a structured questionnaire.

Collection of specimen

Faecal samples were collected in sterile wide mouth containers and labelled accordingly. The samples were submitted to the Research Laboratory of the Department of Medical Microbiology And Parasitology, Faculty of Basic Medical Science, Obafemi Awolowo University, Ile-Ife, Nigeria for processing.

Microscopy analysis and stool culture.

Stool specimens were examined macroscopically for their form and consistency. A faecal smear was made in 0.9% saline and examined for yeast cells on a grease free microscope slide under the x 10 and x 40 objectives. A portion of the stool specimen was aseptically streaked onto Sabouraud Dextrose Agar medium with chloramphenicol(0.5g/l), then incubated at 37^0C for 72 hours.

Candida overgrowth was defined as growth of 105 or more colonies/ml in a pure culture from the liquid stool. From the solid stools, routine inoculation was done to look for growth of a sufficient number of colonies. Representative colonies were picked from a pure culture of *Candida* ($>10^5$ CFU/ml) for further identification. All the suspected colonies were Gram stained and sub-cultured to a CHROM-agar medium for speciation of the isolates. The CD4 T lymphocyte count was determined by a single platform flow cytometer (cytoflowpartec).

Statistical analysis

The Chi-square (χ^2) and Fisher's exact tests (two-tailed) of SPSS version 20 software (SPSS, Inc. Chicago, Illinois) were used to determine the statistical significance of the data. All reported p values were two-sided and a p-value of less than or equal to 0.05 was considered statistically significant.

Results

Patient data and prevalence of candidiasis

A total of 154 HIV patients were investigated for candidiasis comprising 27 males and 127 females (Table 1). The participants were between 18-72 years of age with a mean age of 41 years. Sixty one (39.6%) patients were positive for candidiasis while 93 (60.4%) were negative. Among those that were positive for candidiasis, six (9.7%) were males while 55 (90.2%) were females. The prevalence of candidiasis was significantly higher among those that were females compared with those that were males (P=0.042). The highest number of patients screened and were positive for candidiasis were between the age range of 29-39 (30, 49.2%), followed by 40-50 (19, 31.2%) and 51-61 (11.5%), while those in the age range of 62-72 (2, 3.3%) had the least prevalence of candidiasis. There was no association between the age category of patients and the prevalence of candidiasis (p>0.5).

Table 1: Distribution of Candida colonisation with respect to age and gender

Characteristics	with candidiasis (n=61)	without candidiasis (n=93)	p-value	Odd's ratio	Confidence Interval
Sex			P = 0.042[a]	0.37	0.14-0.91
Male	6 (9.7)	21 (20.3)			
Female	55 (90.2)	72 (77.4)			
Age (Mean)	40.4	41.2			
Age range					
18-28	3 (4.4)	7 (7.5)	P=0.741	0.64	0.14-2.40
29-39	30 (49.2)	35 (37.6)	P=0.156	1.60	0.79-3.25
40-50	19 (31.2)	32 (34.4)	P=0.674	0.86	0.40-1.81
51-61	7 (11.5)	15 (16.1)	P=0.287	0.67	0.22-1.91
62-72	2 (3.3)	4 (4.3)	P=0.748	1.000	0.07-5.46

a=p<0.05 is significant
OR = odd ratio, CI = confidence interval

Relationship between socio-demographic factors, clinical condition, and candidiasis.

Demographic characteristics of HIV patients in relation to candidiasis are shown in Table 2. The two groups were almost the same with regard to occupation, toilet facilities, water supply and the use of ART. There was no relationship between each of the factors and presence of candidiasis (p>0.05). However, among those with different clinical conditions, only those with diarrhoea significantly had a higher prevalence of candidiasis compared with those who did not have diarrhoea (P = 0.001). In addition, patients with candidiasis are seven times likely to have diarrhoea compared with those without candidiasis (OR=7.88; CI=3.27-18.99). In relation to CD4 count of patients, 38 (62.3%) patients with candidiasis had a CD4 count < 200 cells/mm^2 compared with 5 (5%) patients without candidiasis. A significantly higher percentage of those with candidiasis (62.3%) had a CD4 count < 200 cells/mm^2 compared with those without candidiasis (5.4%) (p=0.001). HIV patients with candidiasis are 29 times likely to have a CD4 count < 200 cells/mm^2 compared with those without candidiasis (OR=29.08; CI=9.67-102.39).

Table 2: Association of socio-demographic factors and clinical conditions with candidiasis in HIV patients.

Factors	with candidiasis (n=61)	without candidiasis (n=93)	p-value	Odd's ratio	Confidence Interval
Occupation					
Farmer	3 (4.9)	4 (4.3)	P = 1.000	1.15	0.16-7.07
Trader	29 (47.5)	48 (51.6)	P = 0.621	0.85	0.45-1.61
civil servant	11 (18)	19 (20.4)	P = 0.714	0.86	0.38-1.94
Student	2 (3.3)	2 (2.2)	P = 0.649	1.54	0.11-21.75
Artisan	12 (19.7)	17 (18.3)	P = 0.829	1.09	0.48-2.48
Retiree	2 (3.3)	2 (2.2)	P = 0.649	1.54	0.11-21.75
Jobless	2 (3.3)	1 (1.1)	P = 0.563	3.12	0.16-185.95
Toilet Facilities					
Bush	7(11.5)	7 (7.5)	P = 0.584	1.59	0.45-5.63
Pit	31 (50.8)	44 (47.3)	P = 0.670	1.15	0.61-2.19
Shot put	1 (1.6)	4 (4.3)	P = 0.649	0.37	0.01-3.89
Water closet	22 (36.1)	38 (40.9)	P = 0.551	0.82	0.42-1.58
Clinical conditions					
Nausea	9 (9.7)	13 (14)	P = 0.382	1.07	0.37-2.92
Vomiting	4 (3.2)	9 (9.7)	P = 0.218	0.65	0.14-2.49
Abdominal pain	12 (14.5)	25 (26.9)	P = 0.264	0.67	0.31-1.45
Weight Loss	24 (29)	32 (34.4)	P = 0.350	1.24	0.64-2.40
Appetite	12 (14.5)	6 (6.5)	P = 1.000	3.55	1.14-12.18
Diarrhoea	54 (88.5)	46 (49.5)	P = 0.001[a]	7.88	3.27-18.99
Water Supply					
Pure water	2 (3.2)	7 (7.5)	[b]P =0.320	0.42	0.04-2.30
Well	53 (86.9)	72 (77.4)	P = 0.142	1.93	0.80-4.67
Rain	0 (0)	1 (1.1)	[b]P = 1.000	0.00	0.00-59.46
Spring	0 (0)	1 (1.1)	[b]P = 1.000	0.00	0.00-59.46
Tap	4 (6.6)	8 (8.6)	[b]P = 0.446	0.75	0.16-2.95
Stream	2 (3.2)	4 (5.9)	P = 0.125	0.75	0.16-2.95
CD4 Count					
<200 (cells/mm2)	38 (62.3)	5 (5.4)	P=0.001[a]	29.08	9.67-102.39
>200 (cells/mm2)	23 (37.7)	88 (94.6)			
Antiretroviral therapy (ART)					
With ART	47 (77.1)	77 (82.8)	P = 0.379	0.70	0.32-1.54
Without ART	14 (23)	16 (17.2)			

Distribution of *Candida* species in HIV patients

Sixty-one isolates of *Candida* were recovered from the samples of the subjects. Fifty-six were single strains and five were multiple strains. Sixteen (26.5%) were *C. albicans* while 40 (65.6%) were non-*albicans* species. The non-albicans species included *Candida krusei* (10, 16.4%), *Candida dublinensis* (8, 13.1%), *Candida guilliermondii* (5, 8.2%) and *Candida Parapsilosis* (7, 11.5%). The multiple *Candida* species included *C. albicans* + *C. parapsilosis* (2, 3.3%), *C. albicans* + *C. krusei* (2, 3.3%) and *C. albicans* + *C. tropicalis* (1, 1.6%). *Candida albicans* was the most common yeast isolated (Table 3).

Table 3: Frequency and distribution of various species of *Candida* among subjects

Candida species	Frequency n (%)
Candida albicans	16 (26.3)
C. krusei	10 (16.4)
C. tropicalis	9 (14.8)
C. dublinensis	8 (13.1)
C. guilliermondii	5 (8.2)
C. parapsilosis	7 (11.5)
C. glabrata	1 (1.6)
C. albicans+ C. krusei	2 (3.3)
C. albicans+C. tropicalis	1 (1.6)
C. albicans+C. parapsilosis	2 (3.3)
Total	61 (100)

Distribution of Candida species in HIV patients in relation to therapy

Out of the subjects that had candidiasis, 47 were on ARV while 14 were not. *Candida albicans* (21.3%) was the most predominant species isolated from those that were on ARVs while *Candida krusei* (5, 8.3%) was the most predominant species in patients that were not on ARV. Other species recovered from patients on ARV were: *C. tropicalis* (8, 17%), *C. parapsilosis* (7, 11.5), *C. dubliniensis* (6, 9.8%), *C. guilliermondii* (2, 3.3%) and *C. glabrata* (1, 1.6%). Among those that were not on ARV, were *C. albicans* (3, 4.9%), *C. tropicalis* (1, 1.6%), *C. krusei* (5, 8.2%), *C. guillinermondi* (3, 3.9%), and *C. dublinensis* (3, 3.9%) (Table 4).

Table 4: Species distribution of *Candida* species in HIV-positive patients

Subjects	Species	Number of Isolates	% Number of Isolates
On ARVs (n=47)	*C. albicans*	13	21.3
	C. krusei	5	8.3
	C. albicans+C. krusei	2	3.3
	C. tropicalis	8	17.0
	C. albicans+C. tropicalis	1	1.6
	C. dubliniensis	6	9.8
	C. guilliermondii	2	3.3
	C. albicans+C. parapsilosis	2	3.3
	C. parapsilosis	7	11.5
	C. glabrata	1	1.6
Not on ARVs (n=14)	*C. albicans*	3	4.9
	C. krusei	5	8.2
	C. tropicalis	1	1.6
	C. dubliniensis	2	3.3
	C. guilliermondii	3	3.9

Discussion

This study examined the stool samples of 154 HIV patients for the presence of *Candida* spp. *Candida* overgrowth was detected in 61 (39.6%) HIV patients whose samples were examined, and diarrhoea was significantly associated with candidiasis in the subjects. (P=0.001). This finding is not surprising because many infections occur in HIV/AIDS patients due to suppression of the immune system of which gastrointestinal infection is one of the most common infections. This mostly manifests with symptoms of diarrhoea which result in life-threatening complications.[15] However, a limitation of the study is that subjects without HIV were not included as controls.

The mean age of HIV patients with candidiasis was 40.4 years with an age range of 18-70 years and the age group of 29-39 years was the most commonly affected age group. We observed a significantly higher prevalence of candidiasis among those that were females (55, 90.2%) compared with those that were males (6, 9.7%) (P = 0.042). Similar findings have also been reported by Lar et al.[16] where the carriage rate of candidiasis was found to be more in women than in men. This may be because most men rarely go for routine checkups until the disease becomes symptomatic and during the time the study was carried out only a few men gave their consent.

The low CD4+ T-lymphocyte count has traditionally been cited as the greatest risk factor for candidiasis and current guidelines suggest increased risk once CD4+ T-lymphocyte counts fall below 200 cells/μL.[17] In our study, a CD4 count below 200 cells/mm^2 was a significant risk factor for acquiring candidiasis among HIV patients (P = 0.001). This finding agrees with the reports of Anwar et al.[18] and Esebelahie et al.[19] that observed a significant relationship between low CD4 counts (<200 cells/ml) and candidiasis. Our finding was expected, as immune suppression is a characteristic of HIV infection. This finding underscores the role of cell-mediated immune response as an essential host defence against candidiasis.

Our results showed the evidence of isolation of *Candida* species in the stool of HIV-infected persons on ART and in those not on ART. This indicates that irrespective of whether the patients are on ART or not, they are still predisposed to candidiasis. This does not agree with the reports of most investigators that observed a significant reduction in the prevalence of candidiasis following the use of ART in HIV patients.[20-22] Our findings may probably be due to lack of ART adherence in HIV patients studied.[23]

The incidence of opportunistic infections due to *Candida* spp. has been increasing.[24] *Candida albicans* is one of the most frequently isolated yeasts in clinical laboratories from HIV/AIDS patients.[25] In our study, *Candida albicans* (40, 65.6%) was the most frequently recovered species. This is in line with earlier reports that ranked *C. albicansas* the most frequently isolated yeasts in HIV patients.[19, 26-29] Other *Candida* species recovered included *C. krusei, C. parapsilosis, C. glabrata, C. dublinensis* and C. tropicalis. This spectrum of non-*albicans Candida* species has also been reported by previous investigators.[19,26]

Conclusion

Candidiasis is still an important opportunistic infection in HIV patients in Ile-Ife. Diarrhoea was significantly associated with candidiasis in the subjects. Various species of *Candida* were identified in this study. In view of the findings of this study, there is a need for regular checks for opportunistic infections, including candidiasis in HIV patients to monitor disease progression and prevent subsequent complications such as candidemia and diarrhoea.

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Competing interests

The authors have declared that no competing interests exist.

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