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Oral and Urinary Colonisation of Candida Species in HIV/AIDS Patients in Cameroon

Longdoh A. Njunda[1], Jules C. N. Assob[2], Shey D. Nsagha[3], Henri L. F. Kamga[1], Ejong C. Ndellejong[1], Tebit E. Kwenti[1,*]

[1]Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Buea, P.O. Box 63, Buea, Cameroon
[2]Department of Biomedical Sciences, Faculty of Health Sciences, University of Buea, P.O.Box 63, Buea, Cameroon
[3]Department of Public Health and Hygiene, Faculty of Health Sciences, University of Buea, P.O.Box 63, Buea, Cameroon

Abstract Candidiasis which is one of the most common opportunistic infection in HIV is caused by *Candida* species that vary with respect to their epidemiology and antifungal susceptibility. This study was designed to determine the distribution and antifungal susceptibility pattern in HIV patients in Cameroon. Oropharyngeal and urine specimens were cultured on Sabouraud Dextrose Agar. Speciation was achieved by the germ tube and sugar assimilation tests, and antifungal sensitivity test was performed using a commercially available test kit. 138 (66.67%) of the 207 participants had candidiasis. Among them, oropharyngeal colonisation 122 (81.2%) was significantly (P = 0.0003) higher than urinary colonisation 26 (18.8%). *Candida albicans* was the most predominant species isolated. The prevalence of candidiasis was significantly (P = 0.0002) higher among patients who were not on HAART (Highly Active Antiretroviral therapy) (82.7%) than in patients on HAART (56.4%). No significant association was observed between candidiasis and CD_4+ T cell count. Most fungal isolates were sensitive to Ketoconazole (85.5%) meanwhile most were resistant to nystatin (68.1%). In conclusion, we reported a very high prevalence of candidiasis in HIV patients in Cameroon and the prevalence tended to decrease in individuals on HAART. Ketoconazole was observed to be the most sensitive antifungal agent.

Keywords Candidiasis, HIV, Prevalence, Antifungal, Susceptibility, Oropharyngeal, Urine, HAART, CD_4 + T Cell

1. Introduction

Mucocutaneous candidiasis is one of the most common manifestations of HIV/AIDS worldwide with oropharyngeal candidiasis being the most widely reported[1]. Worldwide, it is estimated that 9.5 million HIV patients suffer from oral candidiasis[2]. Other studies have shown that approximately 90% of HIV/AIDS infected persons have at one point demonstrated oropharyngeal colonisation by *Candida species*[3]. *Candida species* normally colonise the gastrointestinal tract of healthy individuals and in HIV/AIDS patients. The infection is commonly acquired endogenously except in a few cases where strains can be transmitted from person to person[4]. In the course of HIV infection, patients appear to be colonized with one or more dominant strains which can tend not to change over time[5]. Progressive cell mediated immunodeficiency, with CD_4+ T cell counts less than 200cells/µl is a risk factor for colonisation with *Candida* species and development of Candidiasis[6].

Topical or systemic application of antifungal drugs can be used in the treatment of mucocutaneous candidiasis but very often colonisation is not eradicated[5]. Despite the treatment of mucocutaneous candidiasis with oral antifungal agents, increasing evidence shows that prolonged use of these drugs causes resistance to both systemic and topical users[7]. It has been reported that treatment failures have been noticed with azoles, particularly fluconazole used for the treatment of recurrent oropharyngeal candidiasis[8].

In Cameroon, a country in Sub-Saharan Africa, which has an HIV prevalence of 5.3%[9], information on the distribution of *Candida species* in the oral cavity and urine of HIV/AIDS infected population of the country is not readily available. This cross sectional study was therefore designed to determine the rate of *Candida species* colonisation in the oral cavity and the urine of HIV/AIDS patients as well as to determine the antifungal susceptibility profile of the fungal isolates.

2. Materials and Methods

2.1. Study Population

This hospital-based cross sectional study was conducted from the 14th of February to the 15th of August 2011 on

* Corresponding author:
kwentitebit@yahoo.com (Tebit E. Kwenti)
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HIV/AIDS patients recruited in the Mutengene Baptist Hospital. The study was approved by the Cameroon Baptist Convention Institutional Review Board (CBC IRB). Patients of both sexes and all age groups whether on HAART or not were eligible for the study. Patients were required to provide a signed informed consent form which was duly explained to them in the local Pidgin English Language and were not to be on any antifungal medication 3months prior to the recruitment of the participants.

2.2. Sample Collection

Mouth swaps and urine specimens were collected from all participants using sterile cotton wool swabs and wide mouth transparent sterile screw capped containers respectively. Participants were instructed on how to collect mid-stream urine samples.

2.3. Microscopy

Oropharyngeal swab and centrifuged urine sediment samples were microscopically examined on a clean grease free glass slide using the 10X and 40X objectives for the presence of pus cells and of small, round to oval, thin-walled, clusters of budding yeast cells and branching pseudohyphae characteristically typical of *Candida*. Smears were made and Gram stained and latter observed at 100X for fungi elements.

2.4. Culture

Oropharyngeal and urine specimens were cultured on Chloramphenicol impregnated Sabouraud dextrose agar (SDA) and incubated at 35℃ (± 2°C) for 24-72 hours under aerobic conditions for the observation of colonies which are characteristically white to cream coloured, smooth, glabrous yeast-like in appearance. Colonies were Gram stained and sub-cultured on SDA[10].

2.5. Germ Tube Test

Single colonies from sub-cultures were incubated in human serum and incubated at 37℃ and after 24 hours, wet mounts were prepared and examined under the microscope for germ tube formation[10].

2.6. Chlamydospore Formation

All *Candida* isolates from SDA subcultures were tested for the production of chlamydospores in corn meal agar (CMA) incubated at 25℃. Plates were read after 72 hours and examined under the microscope for the presence of chlamydospores.

2.7. Sugar Assimilation

The ability of the *Candida* species to utilize the 12 different sugars (Glucose, Maltose, Sucrose, Lactose, Galactose, Melibiose, Cellobiose, Inositol, Xylose, Raffinose, Trehalose and Dulcitol) present in the commercially available INTEGRAL SYSTEM YEAST Plus (LIOFILCHEM, Italy) was exploited to confirm the identified *Candida* species isolated after incubating the system aerobically at 36 ± 1℃ for 24 - 48 hours.

2.8. CD_4^+ T Cells Counts

CD_4^+ T cells counts was determined from study participants using FASCount® and the counts were categorised according to standards of the WHO, as severe when counts <200 cells/µl; low (200–349cells/µl); moderate (350 – 499cells/µl) and high when counts ≥ 500cells/µl[11].

2.9. Antifungal Susceptibility Testing

The *in vitro* antifungal sensitivity was achieved by testing ten different antifungal agents on all 138 *Candida* species isolated using the microdilution well method in the commercially available INTEGRAL SYSTEM YEAST Plus (LIOFILCHEM, Italy). This kit is a 24 well system containing culture media and dried antifungals for the identification of 32 clinically important yeasts and sensitivity evaluation to antifungals. Sensitivity is evaluated according to growth or inhibition of yeasts after end point minimum inhibitory concentration for antifungal agent is reached in wells containing media and growth indicator. The *in vitro* antifungal susceptibility profile for each *Candida* species was determined by macroscopic observation of colour changes in wells from red (sensitive) to orange (intermediate) to yellow (resistant)

Each test consist of a disposable microtitre plate, which contains dried serial dilutions of 10 antifungal agents; amphotericin B (2µg/ml), nystatin (1.25 µg/ml), fluconazole (64 µg/ml), itraconazole (1 µg/ml), ketoconazole (0.5 µg/ml), 5-fluorocytosine (16 µg/ml), voriconazole (2 µg/ml), econazole (2 µg/ml), miconazole (2 µg/ml) and clotrimazole (1 µg/ml).

2.10. Statistical Analysis

Analysis was done at a 95% confidence interval using SPSS software Version 14.0. Comparative analysis of the prevalence of *Candida* species, impact of HAART, and distribution of different *Candida* species isolated from the oral cavity and urine of HIV/AIDS patients was done using the Chi-square test.

2.11. Limitations

The population of yeast in the oral cavity is highly complex and patients are often colonised by more than one species or strain. Only *Candida* was identified to the species level in this study which is not necessarily reflective of the entire population of yeast present. To identify the entire population of yeast will require techniques that are very expensive and will also require experts to perform the analyses and hi-tech facilities which are not readily available in developing countries.

3. Results

207 participants were recruited into the study. 161 (77.8%) were females and 46 (22.2%) were males (Table 1). The

participants were between 2-63 years of age (mean age of 36years).

Table 1. Age distribution of study participants

Age group	Proportion of total population n (%)
2 – 15	7 (3.4)
16 – 30	69 (33.3)
31 – 45	100 (48.3)
46 – 63	31 (15.0)

A total of 138 (66.67%) participants were colonized by *Candida species*. Among the 138 participants, 112 (81.2%) had oropharyngeal colonisation (OPC) while 26 (18.8%) had urinary colonisation (UC). The prevalence of OPC and UC in the study population was 54.1% and 12.5% respectively. The difference between the prevalence of OPC and UC was significant statistically (P = 0.0003). 19 (9.2%) were positive for both oropharyngeal and urinary *Candida* colonisation (Figure 1).

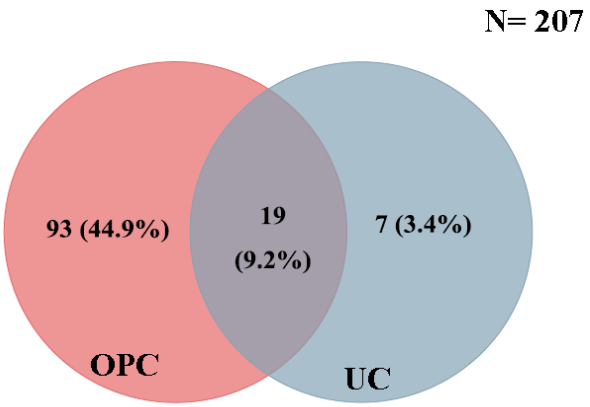

Figure 1. Distribution of candidiasis between the oral cavity and urine of study population

The frequency of OPC and UC was highest in individuals in the age group of 31 – 45 (49.1% and 50% respectively) (Table 2). However, the effect of age on the rate of infection were not observed to be significant statistically (X^2 = 6.325, P = 0.0968). OPC was observed more frequently in females (76.8%) than in male (23.2%). A similar trend was observed with UC (76.9% for females and 23.1% for males) (Table 2). The effect of sex on OPC and UC was not observed to be significant statistically (X^2 = 0.059, P = 0.8080).

Table 2. Distribution of *Candida* colonisation with respect to age and gender

Parameter		OPC *n* (%)	UC *n* (%)	Level of significance
Age range	2-15	3 (2.7)	1 (3.8)	X^2=6.325 P=0.0968
	16-30	34(30.4)	12(46.2)	
	31-45	55(49.1)	13(50.0)	
	46-63	20(17.9)	0(0.0)	
	Total	**112**	**26**	
Gender	Female	86 (76.8)	20 (76.9)	X^2=0.059 P=0.8080
	Male	26 (23.2)	6 (23.1)	

Seven *Candida* species were identified from the study population. *Candida albicans* was the most prevalent species from oropharyngeal (72.3%) and urinary (84.6%) specimen. (Table 3).

Generally, candidiasis was observed more frequently in patients with CD_4^+ T cells counts below 200 cells/µl (Table 4). OPC and UC were observed more frequently in patients with CD_4^+ T cells counts below 200 cells/ µl. The effect of CD_4^+ T cells counts on candidiasis was not observed to be significant statistically (X^2 = 1.568, P = 0.667).

Table 3. Distribution of *Candida* species in oropharyngeal cavity and urine

Species Identified	Site of colonisation	
	OPC *n* (%)	UC *n* (%)
Candida albicans	81 (72.3)	22 (84.6)
Candida tropicalis	19 (17)	4 (15.4)
Candida famanta	4 (3.6)	0 (0.0)
Candida dubliniensis	3 (2.7)	0 (0.0)
Candida krusei	3 (2.7)	0 (0.0)
Candida parapsilosis	1 (0.9)	0 (0.0)
Candida zeylanoides	1 (0.9)	0 (0.0)
TOTAL	**112**	**26**

Table 4. Distribution of *Candida* colonisation by level of immunity

CD_4^+ T cells count category (cells/µl)	OPC *n* (%)	UC *n* (%)	Total
Severe (<200)	33 (29.5)	9 (34.6)	42(30.4)
Low (200-349)	31(27.7)	7(26.9)	38(27.5)
Moderate (350-499)	29(25.9)	8(30.8)	37(26.8)
High ≥500	19 (17.0)	2 (7.7)	21(15.2)
TOTAL	**112 (100)**	**26 (100)**	**138**
Level of significance	X^2= 1.568 P=0.667		

Amongst the 207 participants, 126 (60.9%) were on highly active antiretroviral therapy (HAART), while 81 (39.1%) were not on HAART. A significantly (X^2 = 14.261, P = 0.0002) higher proportion (82.7%) of participants who were not on HAART were colonised by *Candida* species compared to those on HAART (56.4%) (Table 5).

Among participants on HAART, candidiasis was more prevalent in patients with CD_4^+ T cells counts between 350 – 499 cells/µl. But the difference was not found to be significant statistically (X^2 = 3.535, P = 0.3162). Meanwhile among those who were not on HAART, candidiasis was significantly (X^2 = 12.940, P = 0.0048) more prevalent in patients with CD_4^+ T cells counts below 200 cells/µl (Table 6).

OPC was more prevalent among those on HAART (87.3%) than in patients who were not on HAART (74.6%). On the other hand, UC was seen more commonly in individuals not on HAART (65.4%) than in individuals on HAART (34.6%). However the differences in the distribution of OPC and UC in patients on HAART and patients not on HAART were not observed to be significant statistically (X^2 = 5.267, P = 0.1532).

Table 5. Distribution of Candidiasis among patients on HAART and patients not on HAART

Candida colonisation	HAART		Level of significance
	Yes n (%)	No n (%)	
Yes	71(56.4)	67(82.7)	X^2=14.261
No	55(43.6)	14(17.3)	P= 0.0002
Total	**126 (60.9)**	**81(39.1)**	

Table 6. Effect of HAART on *Candida* colonisation stratified according to CD_4^+ T cell category

CD_4^+ T cell range (cell/µL)	HAART			NO HAART		
	OPC *n* (%)	UC *n* (%)	Total (%)	OPC *n* (%)	UC *n* (%)	Total (%)
Severe (<200)	12 (19.4)	1 (11.1)	13 (18.3)	17 (34.0)	8 (47.1)	25 (37.3)
Low (200-349)	15 (24.2)	2 (22.2)	17 (23.9)	15 (30.0)	5 (29.4)	20 (29.9)
Moderate (350-499)	19 (30.6)	5 (55.6)	24 (33.8)	14 (28.0)	3 (17.6)	17 (25.37)
High ≥500	16 (25.8)	1 (11.1)	17 (23.9)	4 (8.0)	1 (5.9)	5 (7.5)
TOTAL	62 (87.3)	9 (12.7)	71	50 (74.6)	17 (25.4)	67

The antifungal susceptibility pattern of clinical isolates of *Candida sp* was determined. In the order of decreasing sensitivity, ketoconazole (85.5%) was the most sensitive drug followed by voricinazole (82.6%) and miconazole (73.9%). The most resistant antifungal drugs were nystatin (68.1%) followed by fluconazole (14.5%) and clotrimazole (12.3%). There was no antifungal drug which did not record any resistance (Table 7).

Candida zeylanoides, Candida parapsilosis and *Candida krusei* which were isolated exlusively from the oral cavity recorded 100% susceptibility to all 10 antifungal agents whereas the other four *Candida* species (*C. albicans, C tropicalis, C. famanta and C. dulbliniensis*) demonstrated some degree of variation in their antifungal susceptobility profile.

C. albicans isolated from the oral cavity reported high susceptibility to voriconazole (81.5%) followed by ketoconazole (80.2%) and econazole (75.3%) but in urine ketoconazole was most susceptible (95.5%) followed by voriconazole (90.9%) and itraconazole (81.8%). Nystatin was the least susceptible antifungal drug to *Candida albicans* in both oropharyngeal and urine samples (Table 8).

In the oral cavity, *Candida tropicalis* recorded high susceptibility to ketoconazole (94.7%) followed by voriconazole (78.9%) and miconazole (73.7%). In urine, the isolates reported absolute susceptibility to ketoconazole (100%) and miconazole (100%). Nystatin was least resistant in both oral cavity and urine samples (Table 9).

All the four *Candida famanta* isolates exhibited 100% susceptibility to ketoconazole, amphotericin B and fluconazole. Resistance was recorded with nystatin (50%) and flucytosine (25%) (Table 10).

Candida dubliniensis exhibited 100% susceptibility to itraconazole, and miconazole, while nystatin was 100% resistant (Table 11).

Table 7. Susceptibility profile of 10 different antifungal drugs tested

Drug type	Susceptibility	OPS *n* (%)	Urine *n* (%)	Total *n* (%)
KCA	Sensitive	93(83.0)	25(96.2)	118(85.5)
	Intermediate	14(12.5)	1(3.8)	15(10.5)
	Resistant	5(4.5)	0(0)	5(3.6)
VOR	Sensitive	91(81.2)	23(88.5)	114(82.6)
	Intermediate	19(17.0)	2(7.7)	21(15.2)
	Resistant	2(1.8)	1(3.8)	3(2.2)
MIC	Sensitive	81(72.3)	21(80.8)	102(73.9)
	Intermediate	21(18.8)	4(15.4)	25(18.1)
	Resistant	10(8.9)	1(3.8)	11(8.0)
ECN	Sensitive	83(74.1)	18(69.2)	101(73.2)
	Intermediate	16(14.3)	7(26.9)	23(16.7)
	Resistant	13(11.6)	1(3.8)	14(10.1)
ITR	Sensitive	78(69.6)	21(80.8)	99(71.7)
	Intermediate	24(21.4)	4(15.9)	28(20.3)
	Resistant	10(9.0)	1(3.8)	11(8.0)
CLO	Sensitive	61(54.5)	15(57.7)	76(55.1)
	Intermediate	37(33.0)	8(30.8)	45(32.6)
	Resistant	14(12.5)	3(11.5)	17(12.3)
FLU	Sensitive	59(52.7)	13(50.0)	72(52.2)
	Intermediate	46(41.1)	9(34.6)	55(39.3)
	Resistant	15(13.4)	5(15.4)	20(14.5)
FCY	Sensitive	75(67.0)	22(84.6)	97(70.3)
	Intermediate	25(22.3)	3(11.5)	28(20.3)
	Resistant	12(10.7)	1(3.8)	13(9.4)
AMB	Sensitive	69(61.6)	16(61.5)	85(61.6)
	Intermediate	40(35.7)	7(26.9)	47(34.1)
	Resistant	3(2.7)	3(11.5)	6(4.3)
NYS	Sensitive	17(15.2)	4(15.4)	21(15.2)
	Intermediate	20(17.9)	3(11.5)	23(16.7)
	Resistant	75(67.0)	19(73.1)	94(68.1)

Table 8. Antifungal susceptibility profile for *Candida albicans*

Infection site	Susceptibility	Antifungals tested									
		KCA *n*(%)	ITR *n*(%)	VOR *n*(%)	ECO *n*(%)	MIC *n*(%)	CLO *n*(%)	FLU *n*(%)	FCY *n*(%)	AMB *n*(%)	NY *n*(%)
OPC	Sensitive	68 (84)	55 (67.9)	66 (81.5)	61 (75.3)	56 (69.1)	44 (54.3)	39 (48.1)	53 (65.4)	45 (55.6)	6 (7.4)
	Intermediate	11 (13.6)	17 (21.0)	12 (14.8)	13 (16.1)	17 (21.0)	28 (34.6)	30 (37.0)	18 (22.2)	34 (42.0)	16 (19.8)
	Resistant	2 (2.5)	9 (11.1)	3 (3.7)	7 (8.6)	8 (9.9)	9 (11.1)	12 (14.8)	10 (12.3)	2 (2.5)	59 (72.8)
UC	Sensitive	21 (95.5)	18 (81.8)	20 (90.9)	14 (63.6)	17 (77.3)	14 (63.6)	10 (45.5)	19 (86.4)	13 (59.1)	2 (9.1)
	Intermediate	1 (4.5)	3 (13.6)	1 (4.5)	7 (31.8)	4 (18.2)	6 (27.3)	8 (36.4)	2 (9.1)	6 (27.3)	3 (13.6)
	Resistant	0 (0)	1 (4.5)	1 (4.5)	1 (4.5)	1 (4.5)	2 (9.1)	4 (18.2)	1 (4.5)	3 (13.6)	17 (77.5)

Table 9. Antifungal susceptibility profile for *Candida tropicalis*

Infection site	Susceptibility	Antifungals tested									
		KCA *n*(%)	ITR *n*(%)	VOR *n*(%)	ECO *n*(%)	MIC *n*(%)	CLO *n*(%)	FLU *n*(%)	FCY *n*(%)	AMB *n*(%)	NY *n*(%)
OPC	Sensitive	18 (94.7)	12 (63.2)	15 (78.9)	12 (63.2)	14 (73.7)	8 (42.1)	11 (57.9)	12 (63.2)	13 (68.4)	5 (26.3)
	Intermediate	1 (5.3)	6 (31.6)	3 (15.8)	2 (10.5)	3 (15.8)	7 (36.8)	6 (31.6)	6 (31.6)	5 (26.3)	3 (15.8)
	resistant	0 (0.0)	1 (5.3)	1 (5.3)	5 (26.3)	2 (10.5)	4 (21.1)	2 (10.5)	1 (5.3)	1 (5.3)	11 (57.9)
UC	Sensitive	4 (100)	3 (75.0)	3 (75.0)	4 (100)	1 (25.0)	1 (25.0)	3 (75.0)	3 (75.0)	3 (75.0)	2 (50.0)
	Intermediate	0 (0)	1 (25.0)	1 (25.0)	0 (0.0)	2 (50.0)	2 (50.0)	1 (25.0)	1 (25.0)	1 (25.0)	0 (0.0)
	Resistant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)

Table 10. Antifungal susceptibility profile for *Candida famanta*

Infection site	Susceptibility	Antifungals tested									
		KCA *n*(%)	ITR *n*(%)	VOR *n*(%)	ECO *n*(%)	MIC *n*(%)	CLO *n*(%)	FLU *n*(%)	FCY *n*(%)	AMB *n*(%)	NY *n*(%)
OPC	Sensitive	4 (100)	3 (75.0)	3 (75.0)	3 (75.0)	3 (75.0)	3 (75.0)	4 (100)	3 (75.0)	4 (100)	1 (25.0)
	Intermediate	0 (0.0)	1 (25.0)	1 (25.0)	1 (25.0)	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)
	Resistant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	2 (50.0)
UC	Sensitive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Intermediate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Resistant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 11. Antifungal susceptibility profile for *Candida dubliniensis*

Infection site	Susceptibility	Antifungals tested									
		KCA *n*(%)	ITR *n*(%)	VOR *n*(%)	ECO *n*(%)	MIC *n*(%)	CLO *n*(%)	FLU *n*(%)	FCY *n*(%)	AMB *n*(%)	NY *n*(%)
OPC	Sensitive	1 (33.3)	3 (100)	2 (66.7)	2 (66.7)	3 (100)	1 (33.3)	0 (0)	2 (66.7)	2 (66.7)	0 (0)
	Intermediate	2 (66.7)	0 (0.0)	1 (33.3)	1 (33.3)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)
	Resistant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	3 (100)
UC	Sensitive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Intermediate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Resistant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

4. Discussion

The overall prevalence of candidiasis among study participants was 66.67%. This result is not very different from the 69.6% reported from a similar study in Italy by Pignato e*t al.*[12]. The prevalence of oropharyngeal colonisation (OPC) in the study population was 54.1%. This result is in line with reports from similar studies in Hong Kong by Tsang and Samaranayake[13], and Taiwan by Hung *et al.,*[6], where prevalences of 54.8% and 52.4% were recorded respectively. The prevalence of urinary colonisation (UC) in this study was 12.5%. Oropharyngeal *Candida* carriage was significantly higher than urinary carriage (P = 0.0003), and this could be explained by the fact that *Candida* is a normal flora of the oral cavity and gastrointestinal tract but not in urine. 19 (9.2%) participants had both OPC and UC.

The prevalence of OPC and UC was highest among individuals between the ages of 31 – 45 years (49.1% and 50% for OPC and UC respectively). However the difference in the prevalence of OPC and UC with respect to age were not found to be significant statistically (P = 0.0968). OPC was observed more in females (76.8%) than in males (23.2%) in this study. Like OPC, UC was also observed more in females (76.9%) than in males (23.1%). However, the prevalence of candidiasis was not observed to be sex dependent (P = 08080). This could be explained by the fact that the majority of participants in this study were females (77.8%).

Candida albicans was the most commonly isolated *Candida* species from both oropharyngeal and urine samples in this study. 72.3% of *Candida albicans* was isolated from the oropharyngeal cavity and this result is in conformity with studies carried out in South Africa (78.6%)[14] and Egypt (78.0%)[15]. In urine, *Candida albicans* recorded a high frequency of 84.6% and this result is similar to that reported by Yongabi *et al.,*[16] in Yaounde, Cameroon, where *Candida albicans* was observed to be the most predominant species in urine. Non- *albicans* species accounted for 27.7% of all *Candida* isolates from the oral cavity. This result is similar to the 24.0% reported in India by Vaishali *et al.*,[17]. The possible reasons why *Candida albicans* constituted a large majority of the *Candida species* isolated in the oral cavity (72.3%) and urine (84.6) could possibly be due to its wide distribution in nature and its possession of multiple adhesins which gives this pathogen the ability to readily colonise host environment by adhering to host mucocutaneous cells, inert polymers, teeth, and salivary molecules[18]. Despite the fact that non-*albicans* species are known to have low colonisation potentials, they cannot be ignored because some of these fungi have been associated with pathology as is the case with *Candida tropicalis* known to be implicated in sepsis associated with bone marrow transplant and oropharyngeal candidiasis in HIV/AIDS as well as head and neck cancer patients[19]. Fungaemia due to *Candida parapsilosis* and *Candida tropicalis* have been reported in neonates[20].

OPC and UC were observed more frequently in individuals with CD_4 T cell count below 200cells/µl. In this study, we failed to observe any significant association between CD_4^+ T cell count and the rate of infection (P = 0.667). This finding is similar to that earlier reported by Schuman *et al.,*[21].

The prevalence of candidiasis was higher among patients who were not on HAART (82.7%) than in individuals who were on HAART (56.4%). This difference was observed to be highly significant (P = 0.0002). This finding is consistent with the observation of Omar[22] in Tanzania who reported *Candida* colonisation to be lower amongst participants on HAART (14.9%) than those not on HAART (38.0%). Among participants on HAART, candidiasis was more prevalent in patients with CD_4 T cell counts between 350 – 499 cells/µl, but this difference was not observed to be significant (P = 0.3162), meanwhile among participants not

on HAART, candidiasis was significantly (P = 0.0048) more common in individuals with CD_4 T cell count below 200cells/µl. This result indicates that HAART has the tendency to reduce the chances of *Candida* colonisation amongst HIV/AIDS patients. These findings are in line with the report of Omar[22] in Tanzania. Several studies have validated the positive role of HAART on immune reconstitution amongst which, Nicolatou *et al.*,[23] in Greece reported a decrease from 58.8% to 37.5% in oral candidiasis amongst HIV/AIDS participants initiated on HAART. Similar trends have been reported in neighbouring Nigeria[24] and in Taiwan[25]. The low prevalence of *Candida* infection amongst HIV/AIDS individuals on HAART could be explained by the fact that antiretroviral therapy substantially prevents occurrence of *Candida* infection in HIV/AIDS patients and its efficacy is being largely attributable to the activity of protease inhibitor enzyme on *Candida* virulence[23].

Among all fungal isolates, ketoconazole was found to be the most sensitive antifungal agent (85.5%). This finding agrees closely with that of a similar study in Iran by Parisa *et al.,*[26]. Other Azoles like miconazole, (73.9%), econazole (73.2%) and itraconazole (71.7%) also recorded good sensitivities' but clotrimazole (55.1) and fluconazole (52.2%) did not demonstrate appreciable susceptibilities. Flucytosine reported a high sensitivity of 70.3%, similar to results obtained from Nigeria by Nweze and Ogbonnaya[27] where a high sensitivity 80.63% was also recorded.

Among the Polyene antifungals, amphotericin B had sensitivity of 61.6% and nystatin 15.2%. These findings are in contrast with reports from Tanzania by Omar[22] who reported 100% susceptibility to both amphotericin B and nystatin among *Candida* isolates. The possible explanation to this disparity could be due to the fact that the participants in this study were infected with *Candida species* which are less sensitive to Polyenes.

Nystatin resistance in this study was 68.1%, the highest recorded amongst the ten antifungal agents tested. These results differ from previous reports from Iran by Parisa *et al.*,[26] who documented no resistance to nystatin. The most probable reason for this marked disparity could be related to intrinsic resistance by *Candida* isolates to nystatin in this study.

The national guideline for the management of HIV/AIDS patients actually recommends the use of nystatin as prophylaxis against *Candida* and other fungi infections[28]. This recommendation was adopted by the Mutengene Baptist Hospital, hence patients were either given nystatin as prophylaxis against fungi infections or it was used in the treatment of *Candida* infection. The prolonged nature in the management of mucosal candidiasis might have caused the development of drug resistant amongst participants in this study. Furthermore, nystatin resistance could have also arisen from the abusive usage as prophylaxis and auto-medication, because among the ten antifungal agents tested, nystatin is the most accessible in terms of cost and availability, hence can easily be obtained over the counter without prescription, giving room for excessive drug abuse.

Among the seven *Candida* species isolated, *Candida albicans* recorded a wider range of resistance amongst the ten antifungal drugs tested as compared to the non-*albicans* species. Meanwhile the non-*albicans* species were less resistant to azoles compared to *Candida albicans* isolates. *Candida tropicalis* being the most frequently isolated non-*albicans* species reported high resistance to clotrimazole (21.7%) and nystatin (56.5%). *Candida famanta* had 50% resistance to nystatin while *Candida dulbliniensis* showed double digit resistance to fluconazole (66.7%) and clotrimazole (33.7). *Candida krusei, Candida parapsilosis* and *Candida zeylanoides* had 0.0% resistance to all ten antifungal drugs. These results are in contrast to a similar study in Nigeria by Nweze and Ogbonnaya[27]. The possible reason for this disparity could be the fact that antifungal susceptibilities have been reported to vary with time, geographical location and drug usage, leading to results varying from one study to another[29].

5. Conclusions

The prevalence of candidiasis among HIV patients in this study was 66.7%, which is very high. Oropharyngeal colonisation was significantly more prevalent than urinary colonisation of candidiasis in this study. The prevalence of candidiasis in this study was not observed to be dependent on the age and sex of the individual. The predominant *Candida species* was *Candida albicans* which was isolated with high frequencies in the mouth and urine of these patients. The rate of infection was lower in individuals who were on HAART than in individuals who were not on HAART, suggesting that HAART has a role to play in lowering the rate of colonisation.

Ketoconazole reported the highest sensitivity rate against all fungal isolates. The other azoles reported mixed sensitivity and resistance but nystatin was predominantly resistant for all fungal isolates in this study.

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