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Original Research Article

The spectrum and enzymatic activities of *Candida* isolates recovered from HIV/AIDS patients in Southeastern Nigeria

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Abstract

Candida is one of the most common opportunistic fungi in HIV/AIDS infection. Successful identification of *Candida* species is important in the treatment and management of all forms of candidiasis. In most parts of Nigeria, especially in the South East axis, the species spectrum of *Candida* yeast colonization in HIV/AIDS is poorly understood. This study was set up to determine the prevalence of oral and blood *Candida* isolates from HIV/AIDS patients in Nigeria. A total of 200 samples comprising of 100 oral swabs and 100 blood samples from HIV/AIDS patients attending Enugu-Ezike district hospital, were analyzed using conventional techniques to identify the presence of *Candida* species. Also, in vitro phospholipase, proteinase and haemolytic activities of the isolates were also carried out. Out of the 200 samples screened, *Candida* species was recovered in 48 (24%) samples. Species recovered included *C. albicans, C. tropicalis, C. parapsilosis, C. guillermondii, C. krusei* and *C. glabrata*. Forty-six isolates were recovered from the oral route while 11 isolates were obtained from the blood, giving a total of 57 isolates. *C. albicans* was the most frequently isolated species from the oral cavity 23 (50%), followed by *C. parapsilosis* 10 (21.7%). The highest incidence of candidiasis among the HIV/AIDS subjects was observed in those aged 21-40 yrs. Phospholipase activity was found in 29 (63%) of the oral isolates while 31 (67.4%) had proteinase activity. Haemolysin activity was observed in 33 (71.7%) isolates. *C. krusei* and *C. guillermondii* isolates did not show any haemolysin activity. Among the blood isolates, 6 (54.4%) had phospholipase activity, while 7 (72.7%) had proteinase activity. Haemolysin activity was observed in 7 (72.7%) isolates. Non- *albicans Candida* species are emerging as potential cause of invasive infection and thus posing a therapeutic challenge in the area investigated.

Key words: *Candida*, HIV/AIDS, Nigeria, Opportunistic infections, Candidiasis

1 Introduction

The human immunodeficiency virus (HIV) is the virus that causes AIDS. It continues to be a major public health concern globally, having claimed the lives of more than 32.7 million people so far [1]. Despite great progress being made in tackling HIV/AIDS epidemic worldwide, its transmission in many parts of the world still shows no sign of abating [2]. At the end of year 2019, approximately 38 million people across the globe were living with the virus, with an estimated 1.7 million individuals, newly infected that same year [1] with between 500,000 – 970,000 people dying from AIDS related illness [3]. Due to debilitated immune system, infected persons are placed at increased risk of several opportunistic infections [4].

Among opportunistic fungal infections in HIV infected patients, candidiasis (caused by *Candida* species) is the most common. Many *Candida* species are harmless commensals or endosymbionts of hosts including humans. However, when there is disruption of mucosal barriers or the individual becomes immunocompromised, they can invade and cause disease [5]. Hence *Candida* species are stringently opportunistic.

The most usually implicated causative agent of both mucosal and systemic *Candida* infections is *Candida albicans.* It is responsible for about 70% of fungal infections around the world [6]. Although *Candida albicans* has been implicated as the most frequently isolated species from HIV infected patients, other *Candida* species such as *C. tropicalis, C. krusei, C. glabarata, C. dubliniensis, C. guillermondii, C. parapsilosis, C. kefyr and C. pellicusa* have also been associated with candidiasis [7]. The clinical significance of these non-albicans *Candida* species lies in the fact that they are usually more resistant to azole antifungal drugs and thus impact effective treatment of patients infected with the fungus [8].

Amongst HIV infected individuals worldwide, oropharyngeal candidiasis is the most common fungal infection (9). The mortality rate of invasive *Candida* infection is close to 40%, despite treatment, especially in clinical conditions [6]. Possible factors that influence candidiasis in HIV positive patients include low CD4 count, high HIV viral load, non-availability or non-usage of highly active antiretroviral therapy [10].

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In Nigeria, especially in the South East and Northern axis, the species spectrum of *Candida* yeast colonization in HIV/AIDS is poorly understood, thus creating a knowledge gap. Isolation of infecting strains of *Candida* is essential because isolates of *Candida* species differ widely both in their ability to cause infection and also in their susceptibility to a wide array of available antifungal agents. The increasing prevalence of multi drug resistance is a global threat to the effective treatment of human diseases of fungal origin. The aim of this study was to determine the prevalence of oral and blood *Candida* isolates from HIV/AIDS patients on antiretroviral therapy in Nigeria. This information has epidemiological relevance and will be useful to clinicians especially those outside Nigeria who treat patients migrating or visiting their countries abroad.

2 Methods

2.1 Study location and sampled population

This study was carried out among HIV/AIDS patients attending Enugu-Ezike District Hospital, Igbo-Eze North Local Government Area, Enugu State, Nigeria. This hospital is a designated HIV/AIDS center and serves several surrounding local governments and the neighboring Kogi and Benue States. Those sampled were either male or female aged 1-80 years. A total of 200 samples was collected in this study, comprising 100 oral samples and 100 blood samples. Samples were collected from 100 patients of different age groups, during the period June to August 2017. The patients were outpatients who visited the hospital twice a week for their antiretroviral therapy (ART). Oral samples were collected using sterile swab sticks by gently rubbing it on the lesioned site while the blood samples were collected using sterile syringes and put into EDTA bottles.

Socio-demographic factors like age, occupation, and locality were obtained from the patients' data as permitted by the ethical committee of the hospital. Samples were labeled and immediately transported at room temperature to the microbiology laboratory at the University of Nigeria, Nsukka for analyses. The blood samples were stored at 4ºC in a refrigerator while the oral samples were stored at room temperature. The samples were all processed within 24 hr.

The sampling approach involved gently rubbing a sterile cotton swab over the lesioned tissue and then subsequently inoculating onto a primary isolation medium (SDA) supplemented with chloramphenicol (0.05g/L).

Blood samples were first processed by inoculating into a biphasic brain-heart infusion agar supplemented with 0.05% sodium polyanethosulfonate and chloramphenicol. Incubation was at 37ºC for 5 days. After 5 days, samples that showed growth were further subcultured on SDA using a sterile wire loop and incubated for 48 hours at 37ºC.

Distinct yeast colonies from each culture plate were subcultured on chromogenic *Candida* agar (Oxoid, Basingstoke, UK) plates and incubated at 37ºC for 2 days. Identification was done as recommended by the manufacturer. An uninoculated agar plate served as the negative control. Subcultures were made from Oxoid chromogenic agar plates onto Sabouraud dextrose agar slants. Slants were incubated at 37ºC, examined for growth after 24 – 48 hr and stored in the refrigerator if they had the desired growth. These slants served as stocks from which subcultures were made for further characterization of the isolates.

2.2 Germ tube test

A light suspension of the 48 hr old yeast isolate was made in 0.5 ml of human serum contained in clean sterile test tube and incubated at 37ºC for 2 – 3 hr. An uninoculated serum sample served as negative control. A loopful of each yeast suspension was placed on a clean glass slide; a coverslip was applied and examined under X40 objective lens for germ tube production.

2.3 Test for virulence factors

2.3.1 Phospholipase Activity

Egg yolk medium was used for this test and this medium consisted of 13.0g Sabouraud's dextrose agar (SDA), 11.7g NaCl, 0.11g $CaCl_2$ and 10% sterile egg yolk (all in 184 ml distilled water). The components were first mixed and sterilized without the egg yolk, then the egg yolk was centrifuged at 500rpm for 10 min at room temperature and 20 ml of the supernatant was added to the sterilized medium. Standard inocula of the test Candida isolates were deposited onto the egg yolk agar medium and left to dry at room temperature. Each culture was then incubated at 37ºC for 48 hr. The assay was conducted in duplicate for each Candida isolate tested.

Phospholipase activity of the isolate was considered positive when a precipitation zone was visible around the colony on the plate. The value of phospholipase activity (Pz) was measured by the ratio of the diameter of the colony to the total diameter of the colony plus the precipitation zone. A Pz value of 1 denotes no activity, and less than one ($Pz < 1$) indicated the phospholipase activity. The lower the Pz value, the higher the enzymatic activity.

2.3.2 Proteinase Activity

Extracellular proteinase activity of Candida isolates was analyzed in terms of bovine serum albumin (BSA) degradation according to the technique described by Ombrella *et al.*, 2012. An 18 hr yeast suspension was standardized, and 10 µL suspension was inoculated onto a 1% BSA medium plate (dextrose 2%, KH_2PO_4 0.1%, $MgSO_4$ 0.05%, agar 2% mixed after cooling to 50°C with 1% BSA solution). The plate was incubated at 37ºC for 5 days. The plates were then fixed with 20% trichloroacetic acid and stained with 1.25% amido black. Acetic acid was used for decolourisation. Degradation of the protein was seen as opaqueness of the agar, corresponding to a zone of proteolysis around the colony which could not be stained with amidoblack. The assay was done in duplicate for each Candida isolate tested. Proteinase activity (Prz) was determined as the ratio of the colony to the diameter of the

proteolytic unstained zone. A Prz value of 1 signifies no activity, and less than one (Prz<1) means proteinase activity. The lower the Prz value, the higher the enzymatic activity.

2.3.3 Haemolytic activity

Haemolytic activity was evaluated with a blood plate assay as described by Manns *et al*., 1994. Media were prepared by adding 7 ml fresh human blood to 100 ml SDA supplemented with glucose at a final concentration of 3% (w/v). A standard inoculum (0.5×10^5) of the test Candida isolates (10 µl) was deposited onto the medium. The plate was then incubated at 37ºC for 48 hr. The ratio of the diameter of the colony to that of the translucent zone of haemolysis (in mm) was used as the haemolytic index (Hz value) to represent the extent of haemolytic activity by different *Candida* isolates. The assay was conducted in duplicate for each yeast isolate tested.

3 Results

3.1 Prevalence of Candida species in the Blood and Oral Cavity

Of the 200 samples screened, 54 (27 for each sample site) were from males while the remaining 146 (73 for each sample site) were from females. Ten (37.03%) of the 27 samples taken from the oral cavity of males yielded growth of *Candida* species while 30 (41.09%) from female oral samples were positive for *Candida* species. For the blood samples, 3 (11.10%) of the 27 samples from males were positive for *Candida* growth while 5(6.85%) of the 73 female samples were also positive for *Candida* species. A total of 57 *Candida* isolates were obtained (Table 1).

3.2 Cultural Characteristics of Candida Species on Selected Media

The *Candida* isolates were identified to the species level. Oxoid Chromogenic *Candida* agar gave precise identification for 6 different *Candida* Species.

Table 1. Frequency of *Candida* species in oral and blood samples of HIV/AIDS patients.

Sample type/Source	No of Sample	*Candida*(+ve) subjects	No of isolates recovered
ORAL			
Male	27	10	11
Female	73	30	35
BLOOD			
Male	27	03	04
Female	73	05	07
TOTAL	**200**	**48**	**57**

Candida albicans was the most frequently isolated species from the oral routes of the HIV/AIDS patients with 23 isolates (50.00%), followed by *C. parapsilosis* which had 10 isolates (21.70%). *C. tropicalis* and *C. guillermondii* were next in terms of frequency of isolation with 4 isolates (8.69%) followed by *C. glabarata* 3(6.50%) and *C. krusei* 2(4.34%) From the blood samples, *C. albicans* and *C. guillermondii* were also the most frequently isolated with 4 isolates each (36.36%), followed by *C. glabrata* 2 (18.18%) and *C. parapsilosis* 1(9.09%). *C. tropicalis* and *C. krusei* were not isolated from the blood samples of the patients (Fig 1). The overall result showed that there was equal number of *Candida albicans* and non-albicans (Fig 4). *Candida* species isolated from the oral tract while the non-albicans *Candida* species were the dominant species isolated the blood of infected patients.

3.3 Phospholipase, proteinase and hemolytic activity of Candida isolates

In vitro analysis of the phospholipase, proteinase and hemolysin activities in the *Candida* isolates were carried out. The distributions of the isolates are shown in Table 3 and 4.

Table 2. Phospholipase, proteinase and hemolytic activity of oral *Candida* isolates.

Isolates	Phospholipase activity IZD (mm) ± SD (Pz VALUE)	Proteinase activity IZD (mm) ± SD (Prz VALUE)	Hemolytic activity IZD (mm) ± SD (Hz VALUE)
OR1	10.2 ± 0.5 (0.58)	9.7 ± 0.5 (0.6)	12.8 ± 0.4 (0.47)
OR2	14.2 ± 0.4 (0.42)	12.2 ± 0.7 (0.49)	13.9 ± 0.3 (0.43)
OR3	NIL (NIL)	13.4 ± 0.6 (0.44)	14.8 ± 0.7 (0.41)
OR4	8.6 ± 0.3 (0.69)	NIL (NIL)	15.4 ± 0.5 (0.39)
OR5	9.4 ± 0.8 (0.64)	8.7 ± 0.6 (0.68)	NIL (NIL)
OR6	NIL (NIL)	14.7 ± 0.3 (0.4)	12.3 ± 0.4 (0.49)
OR7	16.6 ± 0.5 (0.36)	NIL (NIL)	13.7 ± 0.7 (0.44)
OR8	NIL (NIL)	NIL (NIL)	11. 3 ± 0.2 (0.53)
0R9	14.7 ± 0.9 (0.41)	12.4 ± 0.6 (0.48)	14.1 ± 0.6 (0.42)
OR10	7.4 ± 0.6 (0.81)	NIL (NIL	NIL (NIL)
0R11	NIL (NIL)	9.3 ± 0.4 (0.64)	NIL (NIL)
OR12	12.3 ± 0.4 (0.49)	13.6 ± 0.5 (0.44)	NIL (NIL)

Key	Rank
NIL = Negative	0.1 – 0.3 = Strong activity
IZD = Inhibition Zone Diameter	0.31 – 0.6 = Moderate activity
PZ = Zone of Precipitation	0.61 – 0.9 = Low activity

Table 3. Phospholipase, proteinase and hemolytic activity of blood *Candida* isolates.

Isolates	Phospholipase activity. **IZD (mm) ± SD (Pz VALUE)**	Proteinase activity **IZD (mm) ± SD (Prz VALUE)**	Hemolytic activity **IZD (mm) ± SD (Hz VALUE)**
B1	NIL (NIL)	NIL (NIL)	10.2 ± 0.3 (0.59)
B2	11.3 ± 0.4 (0.53)	9.8 ± 0.3 (0.61)	NIL (NIL)
B3	8.7 ± 0.6 (0.69)	NIL (NIL)	NIL (NIL)
B4	NIL (NIL)	7.3 ± 0.4 (0.82)	9.6 ± 0.3 (0.62)
B5	13.3 ± 0.3 (0.45)	8.2 ± 0.5 (0.73)	12.1 ± 0.5 (0.49)
B6	NIL (NIL)	7.8 ± 0.4 (0.77)	NIL (NIL)
B7	9.9 ± 0.5 (0.61)	11.8 ± 0.6 (0.51)	9.0 ± 0.4 (0.66)
B8	NIL (NIL)	9.4 ± 0.6 (0.63)	NIL (NIL)
B9	14.4 ± 0.7 (0.41)	NIL (NIL)	8.4 ± 0.4 (0.71)
B10	NIL (NIL)	7.7 ± 0.3 (0.77)	12.9 ±0.6 (0.47)
B11	11.4 ± 0.8 (0.52)	NIL (NIL)	13.3 ± 0.3 (0.45)

Key	**Rank**
NIL = Negative	0.1 – 0.3 = Strong activity
IZD = Inhibition Zone Diameter	0.31 – 0.6 = Moderate activity
PZ = Zone of Precipitation	0.61 – 0.9 = Low activity
SD	Standard Deviation

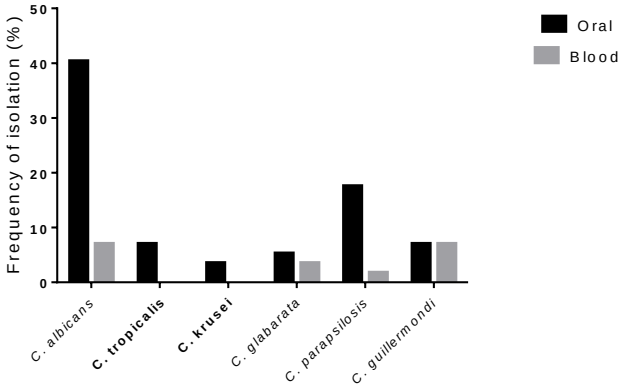

Fig 1. Frequency of isolation of *Candida* spp. from oral and blood samples.

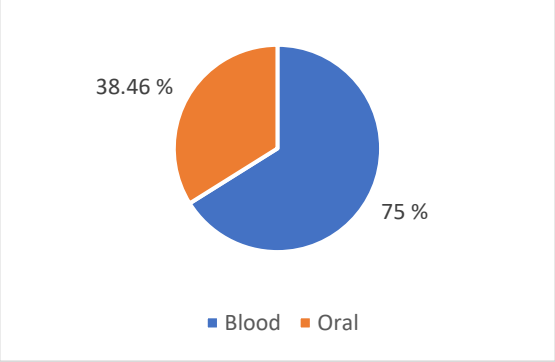

Fig 2. Percentage of sensitivity (%) of *Candida* Isolates from Oral and Blood to Fluconazole

Key: Susceptible ≤ 2 µ/ml; Dose dependent = 4µ/ml and Resistant ≥ 8 µg/ml.

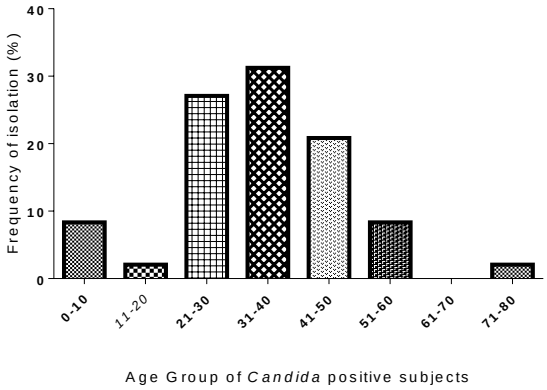

Fig. 3. Frequency of isolation of *Candida* among HIV positive patients of different age groups.

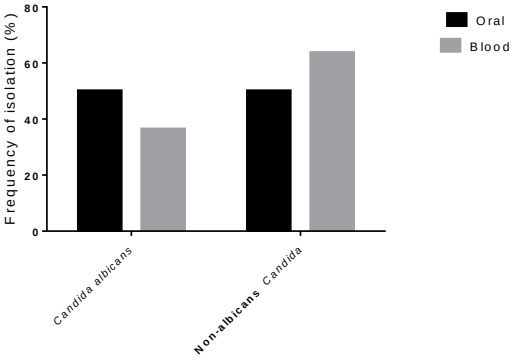

Fig. 4. Frequency of occurrence of *Candida albicans* and non-*albicans Candida* species in the samples.

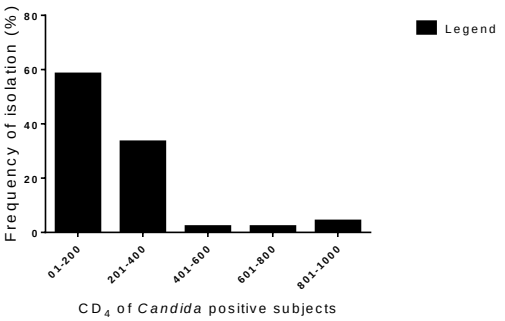

Fig. 5. Relationship between frequency of *Candida* isolation and CD4 count of positive subjects.

4 Discussion

In this study, *Candida* isolates were recovered from blood and oral mucosa of HIV/AIDS patients attending Enugu-Ezike district hospital, Igbo-Eze North Local Government Area, Enugu State, Nigeria. To the best of our knowledge, this is the first time, this kind of study was conducted in the region. The hospital serves as a referral center and provides free HIV testing services with fast and confidential test results. Other services include Early Infant Diagnoses (EID), antiretroviral therapy (including elimination of mother to child transmission), care and support for people living with HIV (including adherence education, counseling, diagnoses and treatment of sexually transmitted infections and opportunistic infections) and diagnoses and

treatment of tuberculosis. Several patients within the local government area, the state and even across the region are usually referred to this facility to assess some of the services. The same activity is obtainable in other referral centers across the country.

The results from our findings suggest that in HIV-infected individuals, oral candidiasis is more implicated than invasive candidiasis. When the immune system of an individual is disrupted, as it happens during HIV/AIDS, the person is placed at an increased risk of a wide variety of opportunistic infections [10]. The findings published by Maheshwari *et al*., 2016 [11] is in agreement with the findings in this study. The authors reported higher number of *Candida* isolates from oral swabs of HIV-infected patients than from blood samples. Other authors reported similar findings [12-13].

The highest frequency of *Candida* isolation was among patients in the age group 31-40 years, followed by 21-30 years, then 41-50 years. Individuals within the age of 21-40 years of age are usually the most affected by HIV infection due to several reasons including sexual risk behaviors, immaturity, economic dependence, feelings of invulnerability and risk appetite [14]. It therefore follows that they presented high *Candida*- positive subjects.

There is higher ($p \leq 0.05$) oral *Candida* prevalence in female subjects than in the male. Approximately 41% of the female oral samples were positive for *Candida* species. This is likely because of the higher number of female HIV-infected patients sampled (73 against 27 for females). It appears that there is a higher rate of HIV infection in women, and this is explained by social, cultural and economic vulnerability of the female gender in the study area [14]. In this current investigation, *Candida albicans*, was the prevalent species among samples from the oral mucosa of the patients with a percentage frequency of 50.00%, distantly followed by *C. parapsilosis* (21.70%), and *C. tropicalis* and *C. guillermondii* with 8.69% frequency of isolation respectively (Figure 1). This dominance by *Candida albicans* has been widely reported in previous studies. For instance, in a study conducted by Mushi *et al*., [15] *Candida albicans* was detected in 119 out of 351 oral swabs (33.90%) collected from HIV-infected patients who visited Care and Treatment Centre (CTC) at Bugando Medical Centre (BMC) in Mwanza City, Tanzania. Mushi *et al*., [15] further reported that non-albicans *Candida* spp. detected were *C. tropicalis* 10 (27.80%), *C. krusei* 9 (25.00%), *C. glabrata* 8 (22.20%) and others 9 (25.00%).

Though *Candida albicans* is the most prevalent species in oral swabs of HIV-infected patients according to several reports from all over the world [9, 10, 16], oral mycobiota varies from one location to another and there is a possibility that *C. albicans* will not always be the predominant species [17].

Non-*albicans Candida* accounted for half of the isolates from the oral routes (Figure 4). This is an indication that they are becoming more implicated in oral candidiasis in HIV/AIDS patients sampled within the study location. This increasing frequency of isolation of non-albicans *Candida* is of concern due to reports of azole-resistance by non-*albicans Candida* [18]. Chances are high that in the near future, the dominant *Candida* species in the oral mucosa of HIV-infected persons in Nigeria could shift from *C. albicans* to non-*albicans Candida*.

Like in the oral samples, *Candida albicans* was the most frequently isolated species from the blood samples of the patients. *C. tropicalis* and *C. krusei* were not recovered at all. As earlier indicated, this is not surprising as *C. albicans* has been repeatedly reported as the most isolated species of *Candida* in various clinical samples in different parts of the world [19]. Besides immunocompromised individuals, *C. albicans* can be detected in about 70% of healthy individuals, depending on the affected tissues and method of sample collection [19]. All these prove the species to be very common in the human body.

Candidiasis manifests as a result of an interaction between host-related factors and yeast-related factors. The yeast-related factors, also referred to as virulence factors, influence the nature of the infection and its severity [20]. The virulence factors in *Candida* help them in invasion, evasion of defense mechanisms of the host and in the establishment of infection. Some of them include adhesion and colonization of the host's mucous membranes and organs, phenotypic and genotypic variation, growth at high temperatures and production of extracellular enzymes [21].

In this study, only a few *Candida* species from the oral routes of the patients were unable to express phospholipase; almost half of the blood isolates failed to produce the enzyme. In general, most of the *Candida* isolates synthesized detectable amounts of phospholipase.

In a related study to evaluate the virulence factors of *Candida albicans* in HIV-positive individuals, Menezes *et al*. [22] observed that 16.2% of the isolates showed high phospholipase activity while 83.3% showed moderate activity, where Pz values of ≤0.63 indicated high activity. Also observed was more intense phospholipase activity for isolates from HIV-infected patients compared to patients without the infection. The report of Back-Brito *et al*. [23] is also in agreement, strengthening the observation that phospholipase is a virulence factor with relatively high expression among *Candida* strains isolated from HIV-infected individuals.

Proteinase is important in the invasion of host tissues by *Candida* spp. due to their ability to break down many substrates of physiological importance, including immunoglobulin, skin proteins and albumin [8]. Most of the isolates from both oral routes and blood evaluated for proteinase activity in this work showed expression of the enzyme.

Proteinase production has been extensively reported for *Candida* especially *C. albicans*. In the earlier cited study of Menezes *et al*. [22], of 75 *Candida* albicans isolates from HIV-positive individuals, 19% showed marked proteinase activity while 81% showed moderate activity where Prz values of ≤0.63 indicated high activity. Similarly, majority of *Candida albicans* isolates from different clinical specimens ranging from 70 to 100% of total isolates expressed proteinases [24-25].

It is thought that the production of haemolysin, the protein responsible for lysing red blood cells, is inherent in *Candida* species and triggered under specific conditions [26]. We

observed haemolytic activity in 72.7% of the blood isolates and 71.7% of the oral isolates. Approximately 91% of the *C. albicans* from the oral mucosa of the patients tested positive for haemolysin production. Elsewhere, all *Candida albicans* isolates from HIV-infected individuals examined by Menezes *et al*. [22] showed haemolytic activity with 80% showing high activity (Hz values below 0.63). Also, Mane *et al*. [8] observed that all 39 *Candida* albicans species isolated from the oral mucosa of HIV-infected individuals were positive for haemolysin production. Reporting on non-albicans *Candida*, Luo *et al*. [27] found that all *C. albicans, C. dubliniensis, C. glabrata, and C. tropicalis* strains isolated from various clinical samples expressed haemolytic factors.

In studying the virulence factors of *Candida* species, haemolytic activity is a fairly recent test and because its methodology is limited by discrepancies in standardization, results from various investigations might not agree [22].

C. albicans has various genetic and phenotypic forms that lead to cryptic mating, copy number variations, polymorphisms, recombination, whole or partial chromosome aneuploidies and sub telomeric hyper variation. Yeasts have the ability to promptly detect environmental changes and adapt its metabolism in order to survive [19]. These attributes help them stay in the host for a very long time and make them the most pathogenic opportunists.

Prophylaxis or antimicrobial therapy encourages resistance among microorganisms by selecting the strains that are able to survive and multiply in the presence of a particular drug [28]. Failure of antifungal treatment could be as a result of factors related to the host (site of infection, immune state, adherence to treatment regimen, formation of abscess, presence of other materials), drug-related factors (pharmacokinetics, administered dose, fungicidal or fungistatic activity) and factors related to the fungus (phenotypic switch, genomic stability, serotype, fungal load, hyphal morphology, biofilm production) [29]. Fluconazole resistance was first observed in strains of *Candida albicans* [30-33]. The fungicidal effect of fluconazole is due to its ability to inhibit the synthesis of ergosterol. All azole antifungals including fluconazole inhibit the function of cytochrome P_{450} system to some level of specificity [34]. Ergosterol is a major component of the fungal cell membrane, therefore, its inhibition causes infeasibility of the cell. The indiscriminate use of this drug has very likely contributed to the rising cases of its resistance among *Candida* species [35]. The MIC of nystatin against the isolates was also evaluated. Most of these isolates were from the oral routes of the HIV-infected individuals because only about 9% of the blood isolates showed resistance to fluconazole. This finding agrees with that of Yee-Chun Chen *et al*., 2003 [36] who reported that only 0.7% of the blood isolates studied showed resistance to fluconazole. Also, Pfaller *et al*., 1999 [37] studied 1579 blood *Candida* isolates, between 1992 and 1998 and discovered that only about 3.3% of these isolates were resistant to fluconazole.

Generally, all the screened patients with high recovery of *Candida* isolates had relatively lower CD4 counts ($p \leq 0.05$) (Figure 6). Approximately 60% frequency of isolation was observed in patients with CD4 count in the range 01-200 cells/µL. Expectedly, candidiasis was higher in patients with weaker immune system. Maheshwari *et al*. [11], made a similar observation where they reported that the mean CD4 T-lymphocyte count in patients with *Candida* infection was 142.6 cells/µL, smaller than 412.3 cells/µL for patients without *Candida* infection. There are a number of other previous studies in which there was a correlation between the occurrence of candidiasis in HIV positive patients and low CD4 counts [38-40]. There is hence enough evidence to suggest that cell-mediated immune response plays a role in the defense of the host against candidiasis [41].

5 Conclusion

Non- *albicans Candida* species are emerging as potential cause of invasive infection and thus posing a therapeutic challenge. There is need for wider surveillance of *Candida* isolates in order to clearly define the exact role of virulent factors and drug resistance in invasive candidiasis.

Competing interests

The authors declare that they have no competing interests.

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