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# Prevalence and antifungal susceptibility pattern of oral candidiasis among HIV-infected patients in a Mission Hospital, southeast Nigeria

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## Abstract:

**Background:** Oral candidiasis is an infection that follows colonization of oral cavity by *Candida* species mostly *Candida albicans.* About 90% of HIV-infected persons develop this disease during the course of HIV infection and could serve as early sign of HIV-related immunodeficiency. Treatment involves the use of antifungal drugs. The objectives of this study are to determine the prevalence of oral candidiasis and the susceptibility of isolated *Candida* species to available antifungal agents among selected HIV-infected patients in a mission hospital, southeast Nigeria.
**Methodology:** This was a descriptive cross-sectional study of 150 consecutively selected HIV-infected patients attending the Heart-to-Heart clinic of Iyi-Enu mission hospital Ogidi, Anambra State, Nigeria, between December 2022 and February 2023. Demographic information of each participant was obtained using structured questionnaire. Five milliliters of whole blood were drawn from the antecubital vein of each participant for $CD4^+$ estimation. Mouth specimens were collected using two sterile cotton swabs for microscopy and culture on Sabouraud Dextrose Agar, and *Candida* species were identified after subculture on CHROMAgar. Antifungal susceptibility testing was performed by Kirby-Bauer disk diffusion method using fluconazole, clotrimazole, ketoconazole, and nystatin disks, and results interpreted according to the guidelines of the Clinical and Laboratory Standards Institute.
**Results:** A total of 98 (65.3%) HIV-infected participants were positive for oral candidiasis, with 4 species of *Candida* isolated; *Candida albicans* (62.2%), *Candida glabrata* (18.4%), *Candida tropicalis* (12.2%) and *Candida krusei* (7.1%). Fifty-nine (60.2%) of the 98 participants had $CD4^+$ cell count < 200, 33 (33.7%) had counts in the range of 200-399, and 6 (6.1%) had counts in the range of 400-499 cells/µL ($p$=0.001). The prevalence of candidiasis was not significantly different between the female (67.0%, 65/97) and male (62.3%, 33/53) participants ($p$=0.6598), but the prevalence was significantly higher ($p$<0.05) in participants age group 21-30 years (80.7%, 42/52), divorced (100%, 1/1) and married (75%, 45/60), those with primary school level education (73.7%, 42/57), civil servants (85.7%, 18/21), and those who performed mouth hygiene once daily (71.9%, 69/96). Nystatin (77.6%, 76/98) showed the highest while fluconazole and ketoconazole (68.4%, 62/98) showed the lowest *in vitro* antifungal activity
**Conclusion:** Oral candidiasis is prevalent among HIV-infected patients in the study population, with evidence of *in vitro* resistance of the *Candida* isolates to available antifungal drugs. Proper diagnosis, susceptibility testing and treatment of infection will be helpful in managing oral candidiasis infection among HIV infected patients.

**Keywords:** oral candidiasis, prevalence, *Candida albicans*, antifungal susceptibility test, HIV patient

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# Prévalence et profil de sensibilité aux antifongiques de la candidose buccale chez les patients infectés par le VIH dans un hôpital de mission, au sud-est du Nigeria

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## Résumé:

**Contexte:** La candidose buccale est une infection qui fait suite à la colonisation de la cavité buccale par des espèces de *Candida*, principalement *Candida albicans*. Environ 90% des personnes infectées par le VIH développent cette maladie au cours de l'infection par le VIH et pourraient constituer un signe précoce d'immunodéficience liée au VIH. Le traitement implique l'utilisation de médicaments antifongiques. Les objectifs de cette étude sont de déterminer la prévalence de la candidose buccale et la sensibilité des espèces isolées de *Candida* aux agents antifongiques disponibles chez des patients sélectionnés infectés par le VIH dans un hôpital de Mission, au sud-est du Nigeria.
**Méthodologie:** Il s'agissait d'une étude transversale descriptive de 150 patients infectés par le VIH sélectionnés consécutivement et fréquentant la clinique Heart-to-Heart de l'hôpital Iyi-Enu Ogidi, État d'Anambra, Nigéria, entre décembre 2022 et février 2023. Informations démographiques de chaque participant a été obtenue à l'aide d'un questionnaire structuré. Cinq millilitres de sang total ont été prélevés de la veine antécubitale de chaque participant pour l'estimation des $CD4^+$. Des échantillons de bouche ont été prélevés à l'aide de deux cotons-tiges stériles pour la microscopie et la culture sur Sabouraud Dextrose Agar, et les espèces de *Candida* ont été identifiées après sous-culture sur CHROMAgar. Les tests de sensibilité aux antifongiques ont été effectués par la méthode de diffusion sur disque de Kirby-Bauer en utilisant des disques de fluconazole, de clotrimazole, de kétoconazole et de nystatine, et les résultats ont été interprétés conformément aux directives du Institut des Normes Cliniques et de Laboratoire.
**Résultats:** Un total de 98 (65,3%) participants infectés par le VIH étaient positifs pour la candidose buccale, avec 4 espèces de *Candida* isolées; *Candida albicans* (62,2%), *Candida glabrata* (18,4%), *Candida tropicalis* (12,2%) et Candida krusei (7,1%). Cinquante-neuf (60,2%) des 98 participants avaient un nombre de cellules $CD4^+ < 200$, 33 (33,7%) avaient un nombre de cellules entre 200 et 399 et 6 (6,1%) avaient un nombre de cellules entre 400 et 499/µL ($p$=0,001). La prévalence de la candidose n'était pas significativement différente entre les femmes (67,0%, 65/97) et les hommes (62,3%, 33/53) participants ($p$=0,6598), mais la prévalence était significativement plus élevée ($p$<0,05) dans participants tranche d'âge 21-30 ans (80,7%, 42/52), divorcés (100%, 1/1) et mariés (75,0%, 45/60), ceux ayant un niveau d'études primaires (73,7%, 42/57), les fonctionnaires (85,7%, 18/21) et ceux qui ont effectué une hygiène buccale une fois par jour (71,9%, 69/96). La nystatine (77,6%, 76/98) a montré l'activité antifongique la plus élevée tandis que le fluconazole et le kétoconazole (68,4%, 62/98) ont montré l'activité antifongique *in vitro* la plus faible.
**Conclusion:** La candidose buccale est prévalente chez les patients infectés par le VIH dans la population étudiée, avec des preuves de résistance *in vitro* des isolats de *Candida* aux médicaments antifongiques disponibles. Un diagnostic, des tests de sensibilité et un traitement appropriés de l'infection seront utiles dans la gestion de l'infection par la candidose buccale chez les patients infectés par le VIH.

**Mots-clés:** candidose buccale, prévalence, *Candida albicans*, test de sensibilité aux antifongiques, patient VIH

## Introduction:

Oral candidiasis is a common infection of the tongue and other oral mucosa caused mostly by *Candida albicans* (1). Other non-*albicans* species of *Candida* that have been implicated in this infection include *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, *Candida guilliermondii*, *Candida parapsilosis*, *Candida pseudotropicalis*, *Candida stellatoidea* and *Candida lusitaniae* (2,3). Oral candidiasis can occur in immune competent (4) as well as in immunocompromised patients (1). *Candida* species colonizes about 40-60% of oral cavity of immune competent individuals and 62-93% of HIV-infected patients (5). It has been reported that more than 90% of HIV patients develop the candidiasis during the course of infection (6), and data shows that about 9.5 million HIV-patients suffer from oral candidiasis (7).

The increased incidence of oral candidiasis in association with HIV/AIDS epidemic attracted the attention of many researchers and has remained the most common opportunistic infection among HIV/AIDS patients (4,8). Oral candidiasis could serve as early sign of HIV related immunodeficiency and is commonly observed in patients with $CD4^+$ counts less than 200 cells/µl (5,8,9). There are many clinical presentations and classifications of oral candidiasis. Vila et al., (4) simply categorized oral manifestations into three broad groups; acute, chronic and chronic mucocutaneous candidiasis syndrome. Several clinical forms can occur in the oral

cavity and in multiple sites at the same time.

Acute pseudomembranous candidiasis also referred to as oral thrush accounts for a third of cases of oral candidiasis (10) and is the most common type among patients with HIV/ AIDS/cancer, and those receiving immunosuppressive therapies (11). It usually presents as multifocal curdy yellow-white plaques throughout the oral mucosa. Chronic and recurrent infection is frequent among HIV/AIDS patients which can progress to esophageal candidiasis with difficulty in swallowing (4).

HIV/AIDS emerged as global crisis since 1981 and its prevalence is still on the increase in many countries especially in the developing ones (12). Sub-Saharan Africa countries bear excessive share of the global HIV burden with 23 million people living with HIV/AIDS in the region (13), out of the estimated 35.3 million people living with HIV/AIDS globally (14). It has been reported that high HIV load, $CD4^+$ count, type of antiretroviral (ART) medication, non-availability or non usage of highly active antiretroviral therapy (HAART) contribute to factors associated with oral candidiasis in HIV patients (7,15). Poor immunologic response by the patients, living in resource limited locales and development of antifungal resistance pose serious problems amongst the patients (16).

Infection with *Candida* has been related with some virulent factors. These include its ability to attach to the epithelial cell walls, persistent within epithelial cells, induction of tumor necrosis factor, proteinases, ability to form mycelia, germ tube formation (11), and morphological switching (4). A major biological characteristic of *C. albicans* is its ability to form pathogenic biofilms which is an important factor in its ability to cause infection (17,18). Once biofilm is formed, the expression of *Candida* virulence factors increases and its susceptibility to antimicrobials and phagocytosis decreases exceedingly (19).

Oral candidiasis can be diagnosed by identifying clinical signs and symptoms, direct examination of oral sample smear for the presence of *Candida* species, positive culture and serological tests (20). Treatment involves the use of antifungal agents like azoles (clotrimazole, itraconazole, miconazole, ketoconazole, fluconazole), polyenes (nystatin and amphoteri cin B), 5-flourocytosine, allylamineestrocarbonates, morpholine, and caspofungin (21). The choice of antifungal drug depends on the type of lesion and the patients' immunological status. The antifungal agents target the cell membrane, cell wall and nucleic acids of *Candida*. HIV/AIDS patients are usually treated topically as well as systemically. Patients with relapsing candidiasis are treated with antifungals that have the least risk of resistance development or selection of resistant strains (22). The widespread use of these antifungal agents has led to an increase in antifungal resistance. Azole drugs especially fluconazole have been implicated greatly in the development of resistant strains (23).

Oral candidiasis in HIV/AIDS patients remains a major cause of morbidity, and negatively affect the quality of life of such persons. Controlling opportunistic *Candida* species associated with oral candidiasis through proper species identification and susceptibility testing of isolates to common antifungal drugs before administration remains the best way of managing infected individuals. The objective of this study therefore, is to determine the prevalence of oral candidiasis among HIV-infected patients and the susceptibility of isolated *Candida* species to the available antifungal agents in a Mission Hospital, southeast Nigeria.

## Materials and method:

**Study design:**

This is hospital-based descriptive cross-sectional study conducted at the Heart-to-Heart clinic in Iyi-Enu mission hospital Ogidi, Anambra State, Nigeria, from December 2022 to February 2023 (3 months). The Heart-to-Heart clinic of the hospital is a clinic that manages HIV/AIDS outpatients and inpatients.

**Ethical clearance:**

Informed consent of the study participants and the approval of the ethical committee of Iyi-Enu Mission Hospital were obtained.

**Study participants:**

Included in the study are male and female participants aged ≥ 15 years with positive HIV status, who have not taken any antifungal drug within two weeks prior to enrolment and sample collection.

**Sample size and participant selection:**

The sample size was estimated using the Leslie Kish formula (24), which gave the calculated minimum number of participants as 150, who were then recruited using consecutive sampling method over the period of study.

**Socio-demographic and clinical data collection:**

A structured questionnaire was used to collect information on gender, age, marital status, educational level, socio economic status,

oral hygiene care and length of HIV infection of the participants.

**Specimen collection:**

Five milliliter (5mls) of whole blood was drawn from the antecubital vein of each participant into EDTA anti-coagulated bottle for $CD4^+$ estimation. Oral swabs were also collected from each of the participant using two sterile cotton swabs. The specimens were transported in an ice pack to the laboratory of the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka for analysis.

**$CD4^+$ cell estimation:**

The $CD4^+$ count was determined using the Partec Cytoflow Counter II (Partec Germany) and correlated with presence or absence of candidiasis. The counts were categorized according to WHO guideline as severe (< 200 cells/µL), low (200-349 cells/µL), moderate (350-499 cells/µL) and high (≥500 cells/µL) (25).

**Microscopy and culture of swab samples:**

Swab samples were examined microscopically on clean grease free glass slide using 10x and 40x objective lens for presence of pus cells, oval to round clusters of budding yeast cells and branching pseudohyphae (7).

The second cotton swab was aseptically inoculated onto Sabouraud Dextrose Agar (SDA) impregnated with 0.05mg/ml chloramphenicol and incubated aerobically at 35-37$^o$C for 24 hours for observation of colonies (26). Thereafter, discrete colonies were emulsified in 1ml sterile distilled water and a loopful inoculated on CHROMAgar (Biotech, India) medium. This was incubated for 24 hours at 25$^o$C for identification of various species of *Candida* based on color, with light green for *C. albicans*, cream color for *C. glabrata*, pale pink for *C. krusei* and dark blue for *C. tropicalis* (27,28).

**Antifungal sensitivity test:**

Susceptibility testing was carried out on the *Candida* isolates using the Kirby-Bauer disk diffusion method and interpretation according to the Clinical and Laboratory Standards Institute guidelines (29). The antifungal drugs tested include; fluconazole (25µg), clotrimazole (10µg), ketoconazole (10µg) and nystatin (50 µg) (Rosco diagnostic company, Neosensitabs, Denmark).

Twenty-four hours old inoculum suspension of each *Candida* isolate was prepared and adjusted to the turbidity of 0.5 McFarland standards, and then seeded on sterile Mueller Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue. The plate was dried at ambient temperature for 15min, and the discs containing the different antifungal agents were aseptically placed on the agar lawn. The agar plate was incubated for 24 hrs at 35$^o$C. The diameter of zone of inhibition was measured in millimeter. The AST was carried out on each isolate in duplicate and the mean value was used for susceptibility interpretation as sensitive, moderately sensitive and resistant in accordance with CLSI and the manufacturers guidelines.

**Statistical analysis:**

Data were described in frequency and percentages. The Pearson Chi-square test was used to determine association of oral candidiasis with gender, age, marital status, education level, occupation, oral hygiene and length of HIV infection. P value < 0.05 was considered statistically significant at 95% confidence interval.

## Results:

The demographic characteristics of the 150 HIV-infected participants is shown in Table 1. Majority of the participants were females (64.7%). Fifty-two (34.7%) participants are in the age group 21-30 years and 16.0% are ≤ 20 years of age. Most are single (59.3%) while 0.7% are divorced. Seventy-one (47.3%) had secondary level education, 37.3% are traders while 14% are civil servants.

Of the 150 HIV-infected participants, 98 (65.3%) had oral candidiasis, with 4 species of *Candida* identified; *C. albicans* (62.2%), *C. glabrata* (18.4%), *C. tropicalis* (12.2%), and *C. krusei* (7.2%) (Table 2). Fifty-nine (60.2%) of the 98 HIV-infected patients with oral candidiasis had $CD4^+$ count <200 cells/µl, 33 (33.7%) had their $CD4^+$ cell count in the range 200-349 cells/µl while 6 (6.1%) had theirs in the range 350-499 cells/µl. There was a statistically significant association between $CD4^+$ cell count and oral candidiasis ($p$=0.001).

The prevalence of oral candidiasis with respect to socio-demographic and clinical data is as presented in Table 3. The prevalence of oral candidiasis of 67.0% (65/97) in the female was higher than 62.3% (33/53) in the male participants, but this rate was not significantly different ($p$=0.6598). However, the prevalence of oral candidiasis was significantly higher in participants age group 21-30 years (80.7%, 42/52) compared to other age groups ($p$= 0.008), and also in divorced (100%, 1/1) and married (75%, 45/60) participants ($p$=0.028),

in participants with primary school level education (73.7%, 42/57, $p$=0.0014), civil servants (85.7%, 18/21, $p$=0.0482), and in participants who performed mouth hygiene once daily (71.9%, 69/96, $p$=0.0388). The prevalence of oral candidiasis was not significantly associated with duration of HIV infection when analysed with Chi square test for trend ($x^2$=0.4882, $p$= 0.4847) (Table 3).

The antifungal susceptibility pattern showed that *C. albicans* was most susceptible to all the four antifungal drugs while *C. krusei* was the most resistant. Nystatin (77.6%, 76/ 98) was the drug with the highest *invitro* antifungal activity while fluconazole and ketoconazole (68.4%, 62/98) had the lowest *in vitro* antifungal activity (Table 4).

Table 1: Socio-demographic and clinical characteristics of HIV-infected participants in a Mission Hospital, southeast Nigeria

| Characteristics | Frequency | Percentage (%) |
|---|---|---|
| **Gender** | | |
| Female | 97 | 64.7 |
| Male | 53 | 35.3 |
| **Age group (years)** | | |
| <20 | 24 | 16.0 |
| 21-30 | 52 | 34.7 |
| 31-40 | 41 | 27.3 |
| >40 | 33 | 22.0 |
| **Marital status** | | |
| Single | 89 | 59.3 |
| Married | 60 | 40.0 |
| Divorced | 1 | 0.7 |
| **Educational level** | | |
| Primary | 57 | 38.0 |
| Secondary | 71 | 47.3 |
| Tertiary | 22 | 14.7 |
| **Occupation** | | |
| Students | 41 | 28.0 |
| Traders | 57 | 37.3 |
| Civil servants | 21 | 14.0 |
| Self-employed | 31 | 20.7 |
| **Oral hygiene practice** | | |
| Brush teeth once daily | 96 | 64.0 |
| Brush teeth twice daily | 54 | 36.0 |
| **Duration of HIV infection (months)** | | |
| ≤6 | 19 | 12.7 |
| 7-24 | 36 | 24.0 |
| 25-35 | 44 | 29.3 |
| ≥36 | 51 | 34.0 |

Table 2: Frequency of *Candida* species isolated from HIV-infected participants in a Mission Hospital, southeast Nigeria

| Candida species | Frequency | Percentage (%) |
|---|---|---|
| *Candida albicans* | 61 | 62.2 |
| *Candida glabrata* | 18 | 18.4 |
| *Candida tropicalis* | 12 | 12.2 |
| *Candida krusei* | 7 | 7.2 |
| **Total** | **98** | **100.0** |

Table 3: Prevalence of oral candidiasis in HIV-infected participants in a Mission Hospital, southeast Nigeria with respect to socio-demographic and clinical characteristics

| Characteristic variables | No tested | No positive (%) | $x^2$ | OR (95% CI) | *p* value |
|---|---|---|---|---|---|
| **Gender** | | | | | |
| Female | 97 | 65 (67.0) | 0.1635 | 1.231 (0.6123-2.475) | 0.6598 |
| Male | 53 | 33 (62.3) | | | |
| **Age group (years)** | | | | | |
| ≤20 | 24 | 12 (50.0) | 11.842 | NA | 0.008* |
| 21-30 | 52 | 42 (80.7) | | | |
| 31-40 | 41 | 21 (51.2) | | | |
| >40 | 33 | 23 (69.7) | | | |
| **Marital status** | | | | | |
| Single | 89 | 52 (58.4) | 4.851 | NA | 0.028* |
| Married | 60 | 45 (75.0) | | | |
| Divorced | 1 | 1 (100.0) | | | |
| **Educational level** | | | | | |
| Primary | 57 | 42 (73.7) | 13.091 | NA | 0.0014* |
| Secondary | 71 | 49 (69.0) | | | |
| Tertiary | 22 | 7 (31.8) | | | |
| **Occupation** | | | | | |
| Students | 41 | 21 (51.2) | 7.896 | NA | 0.0482* |
| Traders | 57 | 37 (64.9) | | | |
| Civil servants | 21 | 18 (85.7) | | | |
| Self-employed | 31 | 22 (71.0) | | | |
| **Oral hygiene practice** | | | | | |
| Brush teeth once daily | 96 | 69 (71.9) | 4.268 | 2.203 (1.098-4.418) | 0.0388* |
| Brush teeth twice daily | 54 | 29 (53.7) | | | |
| **Duration of HIV infection (months)** | | | | | |
| ≤6 | 19 | 7 (36.8) | $0.4882^+$ | NA | 0.4847 |
| 7-24 | 36 | 26 (72.2) | | | |
| 25-35 | 44 | 37 (84.1) | | | |
| ≥36 | 51 | 29 (56.9) | | | |

$x^2$= Chi square; OR = Odds Ratio; CI = Confidence Interval; * = statistically significant; + = Chi square by trend; NA = Not Applicable

## Discussion:

Oral candidiasis continues to pose a major problem in HIV-infected individuals especially in sub-Saharan Africa where about 23 million people live with HIV/AIDS (15). Mahajan et al., (6) reported that more than 90% of HIV patients develop oral candidiasis during the period of infection. It could serve as early sign of HIV related immunodeficiency and is often observed in patients with $CD4^+$ counts less than 200 cells/µl (5,8,9). As observed in this study, HIV participants with $CD4^+$ count < 200 cells/µl had oral candidiasis prevalence of 60.2% while those with $CD4^+$ counts in the range of 350-499 cells/µl had 6.1%, with a significant association ($p$=0.001) between oral candidiasis and $CD4^+$ count. Our finding agrees with the reports of Ambe et al., (7), Berberi et al., (30), Holiwala et al., (31) and Erfaningejad et al., (32) who in their various studies reported significant associations between $CD4^+$ cell count < 200 cells/µl and high prevalence of oral candidiasis.

A wide range of prevalence of oral candidiasis (11-90%) have been reported among HIV/AIDS patients (7,29,33). The high prevalence of 65.3% reported in our study agrees with prevalence rates of 60.0%, 69.0% and 65.5% reported by some researchers respectively (33,34,35). While higher prevalence of 80% and 79.4% were reported in Cote d'Ivoire (28) and Ghana (36), some other researchers have reported lower prevalence rates of 29-50% (5,7,32,37,38), and prevalence as low as 9.7% have also been reported in Jos, Nigeria (39). These differences in prevalence of oral candidiasis among HIV patients could be as a result of geographic areas sampled, cultural behaviors and the diagnostic methods used.

*Candida albicans* accounted for 62.2% of the *Candida* species identified in this study, while non-*albicans Candida* such as *C. glabrata* (18.4%), *C. tropicalis* (12.2%) and *C. krusei* (7.2%) accounted for 37.8%. The isolation of *C. albicans* as the most prevalent species may be associated with its many pathogenic factors (32), and its ability to cause infection with low colony count (12). The high prevalence of *C. albicans* in our study agrees with the results of other researchers (7,31,37,40,41) but higher prevalence of ≥ 80% has been reported in South Africa (42), Ghana (28) and Ivory Coast (36).

Table 4: Antifungal susceptibility profile of the oral *Candida* species isolated HIV-infected participants in a Mission Hospital, southeast Nigeria

| ***Candida* species** | **Susceptibility** | **Antifungal drugs (%)** | | | |
|---|---|---|---|---|---|
| | | **Fluconazole** | **Ketoconazole** | **Nystatin** | **Clotrimazole** |
| *Candida albicans* (n=61) | Sensitive | 47 (77.0) | 47 (77.0) | 52 (85.3) | 50 (82.0) |
| | Moderately sensitive | 5 (8.2) | 4 (6.6) | 1 (1.6) | 3 (4.9) |
| | Resistant | 9 (14.8) | 10 (16.4) | 8 (13.1) | 8 (13.1) |
| *Candida glabrata* (n=18) | Sensitive | 6 (33.3) | 7 (38.9) | 10 (55.6) | 8 (44.4) |
| | Moderately sensitive | 1 (5.6) | 2 (11.1) | 1 (5.6) | 2 (11.1) |
| | Resistant | 11 (61.1) | 9 (50.0) | 7 (38.8) | 8 (44.4) |
| *Candida tropicalis* (n=12) | Sensitive | 6 (50.0) | 6 (50.0) | 9 (75.0) | 7 (58.3) |
| | Moderately sensitive | 2 (16.7) | 2 (16.7) | 0 | 2 (16.7) |
| | Resistant | 4 (33.3) | 4 (33.3) | 3 (25.0) | 3 (25.0) |
| *Candida krusei* (n=7) | Sensitive | 3 (42.9) | 2 (28.6) | 5 (71.4) | 2 (28.6) |
| | Moderately sensitive | 0 | 0 | 0 | 4 (57.1) |
| | Resistant | 4 (57.1) | 5 (71.4) | 2 (28.6) | 1 (14.3) |
| **Total (n=98)** | Sensitive | 62 (63.3) | 62 (63.3) | 76 (77.6) | 67 (68.4) |
| | Moderately sensitive | 8 (8.2) | 8 (8.2) | 2 (2.0) | 11 (11.2) |
| | Resistant | 28 (28.5) | 28 (28.5) | 20 (20.4) | 20 (20.4) |

*Candida tropicalis*, *C. glabrata* and *C. parapsilosis* have emerged as *Candida* species of health concern over the past few decades and cause disease in 20-40% of immunocompromised persons (7). Their emergence can be attributed to improved identification methods, type of patients' disease and antifungal drugs used (43). In our study, *C. glabrata* (18.4%) was the most frequent non-*albicans Candida* isolated, which agrees with the findings of Erfaninejad et al., (32), Ewumwem et al., (37) and the meta-analysis review done in sub-Saharan Africa over a period of 10 years (44). In contrast, higher prevalence of *C. tropicalis* have been reported in Brazil (45) and China (46). Factors such as sample size, immune status and life style of patients, oral hygiene and high-risk behaviors may contribute to the differences in *Candida* species isolated and their prevalence (40).

As observed in our study, the prevalence of oral candidiasis was higher in female (67.0%) than in male (62.3%) HIV participants but this was not statistically significant ($p$= 0.6598). Our observation supports the reports of higher prevalence in females by other researchers (34,36,40,46). The lower prevalence in males may be attributed to their apparent low interest in attendance of health clinics for HIV testing and counseling until the disease becomes symptomatic (40). Ignorance of HIV infection, and the mythical belief in southeast Nigeria that young men who prosper in business ventures are usually mystically killed through native poisoning, may have created fear in these men that lead them to not voluntarily test for their HIV status early in the course of HIV infection. Contrarily, Suryana et al., (5) reported significantly higher prevalence of oral candidiasis in HIV infected males.

Oral candidiasis occurred significantly more in HIV-infected patients in age group 21-30 years (80.7%, $p$=0.008) in our study. This age group represents sexually active youths and it has been suggested that lack of awareness of the risk factors associated with HIV and opportunistic infections may have predisposed them to oral candidiasis (34). However, other researchers have reported higher prevalence of candidiasis among age group ≥ 30 years (5,

30,37). Analysis of the prevalence of oral candidiasis with respect to level of education showed that the prevalence was significantly higher among HIV participants with primary school level education (73.7%), which may be attributed to apparent low level of personal and oral hygiene in this group of poorly educated people. The prevalence was also significantly higher among civil servants (85.7%) and self-employed (71.0%) than other occupational groups ($p$=0.0482). The reasons for the higher prevalence of oral candidiasis in these occupational groups of HIV-infected patients is not clear.

Our study also reported significantly higher prevalence of oral candidiasis among HIV patients who perform oral hygiene (tooth brushing) only once a day (71.9%) compared to those who performed it twice a day (29.6%) ($p$=0.0388). This observation agrees with the study of Wang (7), who also reported high prevalence of candidiasis among patients who brushed once daily, implying that good oral hygiene will be an important means of managing oral candidiasis. Oral candidiasis can be acquired at any stage of HIV infection and could serve as early sign of HIV related immunodeficiency (5,8). Although, the prevalence of oral candidiasis was higher among participants who have had HIV infection for a period of 25-35 months (84.1%) and 7-24 months (72.2%), there was no significant difference on Chi square test for trend analysis ($x^2$=0.4882, $p$= 0.4747) between length of infection of HIV infection and oral candidiasis, indicating that length of time of HIV infection is not a factor in acquiring oral candidiasis.

The widespread use of antifungal drugs has increased the rate of antifungal resistance by *Candida* species which poses a major challenge in the treatment and management of oral candidiasis (47). The antifungal susceptibility pattern carried out in our study showed that *C. albicans* was the most susceptible to all the antifungal drugs tested while *C. krusei* was the most resistant. This result agrees with study of Wang (7), who also reported *C. albicans* as the most sensitive and *C. krusei* and *C. glabrata* as most resistant to seven antifungal drugs tested in his study. *Candida glabrata* and *C. krusei* have been reported to be less susceptible to antifungal drugs and infections caused by them tend to be more difficult to treat (47). Of the four antifungal agents tested in our study, nystatin (77.6%) was the most active *in vitro* while fluconazole (63.3%) and ketoconazole (63.3%) were the least active. This observation agrees with the results of Wang (7) and Hodiwala et al., (31), who in their separate studies reported nystatin as the most active antifungal agent against oral *Candida* isolates. In contrast, a research study in Cameroon (48) reported ketoconazole (85.5%) as the most active and nystatin (68.1%) as the least active of the ten antifungal drugs tested. In a systematic review and meta-analysis of comparative efficacy of antifungal agents used in the treatment of oral candidiasis among HIV-infected adults in 2021 (49), fluconazole was ranked the most effective antifungal against oral *Candida.*

The emergence of antifungal resistant *C. albicans* and non-*albicans Candida* is of serious concern to healthcare workers all over the world (46). In our study, the resistance rate of *Candida* species to fluconazole and keto -conazole is 28.6% which agrees with 24.6% and 24.0% resistance rates to fluconazole reported by Wang (7) and Osaigbovo et al., (47) respectively. Resistance rate as high as 90.0% have also been reported to flucona- zole by *C. albicans* (32). Fluconazole is usually the first line treatment agent of choice because of its favorable pharmacokinetic profile, low toxicity, availability, minimal drug interaction and minimal adverse effects (50), but *Candida* strains resistant to fluconazole have recently emerged (41). The antifungal drugs tested in our study can be obtained over-the-counter in Nigeria, a practice that has encouraged self medication and may have contributed to the resistance of *Candida* species observed in our study.

## Conclusion:

Oral candidiasis was highly prevalent among HIV-infected participants in this study. Age group, marital status, educational level, occupation, and oral hygiene are significant factors associated with oral candidiasis in por HIV-infected participants. Nystatin was the most active *in vitro* antifungal drug tested, but emergence of non-*albicans Candida* is also of great concern. Proper identification of *Candida* species and susceptibility testing to antifungal drugs before administration will be helpful in managing oral candidiasis among HIV-infected patients.

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## Contributions of authors:

CCE conceived and designed the study, and wrote the manuscript, CJN collected and processed the samples, CGA, NVA and ANM carried out the literature search and critical review of the manuscript, CMO perform statistical analysis of the data and reviewed the manuscript. All authors read and approved the final manuscript.

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