

**PREVALENCE AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF  
ORAL CANDIDIASIS ISOLATED FROM HIV-POSITIVE PATIENTS IN  
BENIN-CITY**

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL  
LABORATORY SCIENCE, UNIVERSITY OF BENIN, IN PARTIAL  
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**OCTOBER, 2024.**

## CERTIFICATION

This is to certify that this seminar work was carried out by **ADEBOLA SAMUEL ATURUKA** with the matriculation number **BMS1902202** under the supervision of **DR (MRS) I. M. MOSES-OTUTU** in partial fulfilment of the requirements for the award of Bachelor of Medical Laboratory Science (BMLS) from the University of Benin, Benin City, Edo State, Nigeria.

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## **DEDICATION**

This work is dedicated to God Almighty who has led and helped me this far.

Ebenezer!

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I give thanks to almighty God, my creator who has granted me grace and strength to finish this seminar work.

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## ABSTRACT

Oral candidiasis remains a significant opportunistic infection among HIV-infected individuals, often serving as an early marker of immune suppression. This study aimed to determine its prevalence and assess the antifungal susceptibility patterns of *Candida* species isolated from HIV-positive patients attending a secondary health facility in Benin City, Nigeria. Sterile cotton swab was used to collect oral specimens from each participant which were gram-stained for microscopic examination. Positive samples were then cultured and subjected to sensitivity test. 250 participants were screened, 76 (30%) yielded positive for oral candidiasis. Among the sociodemographic factors analyzed, only age showed a significant association with the prevalence of oral candidiasis ( $p < 0.001$ ). The highest occurrence of oral candidiasis was observed in patients above 50 years. Antifungal susceptibility testing using the Kirby-Bauer disk diffusion method revealed varied resistance patterns. Amphotericin B showed the highest resistance rate (30.4%), while nystatin had the highest sensitivity rate (26%). Resistance to fluconazole, a commonly used antifungal, was also notable at 19.6%, raising concerns about self-medication and antifungal misuse in the region. Despite widespread HAART coverage, the prevalence of oral candidiasis highlights gaps in treatment adherence and monitoring. The findings emphasize the need for regular oral health assessments, targeted antifungal therapy based on susceptibility testing, and health education to improve HAART adherence and oral hygiene. These measures are crucial in reducing the burden of oral candidiasis in HIV-infected populations.

## CHAPTER ONE

### 1.0

### INTRODUCTION

#### 1.1 BACKGROUND OF THE STUDY

HIV remains a critical public health issue worldwide, especially in sub-Saharan Africa, where the disease burden is disproportionately high (UNAIDS, 2021). The virus progressively weakens the immune system by attacking CD4+ T-helper cells, crucial for maintaining immune responses. If left untreated, HIV can develop into AIDS, characterized by severe immune system failure, leaving individuals vulnerable to a range of opportunistic infections (WHO, 2022). Opportunistic infections are a major cause of illness and death in HIV-positive individuals, as their immune systems are too compromised to fend off infections that would not normally affect healthy individuals (Okoh *et al.*, 2020).

Among the numerous opportunistic infections affecting people with HIV, oral candidiasis stands out as one of the most common and serves as an early warning sign of immune system deterioration. Caused primarily by *Candida albicans*, and sometimes by other species like *Candida glabrata* and *Candida tropicalis*. Oral candidiasis is a fungal infection that typically affects the mouth and throat (Akpan *et al.*, 2021). This condition presents as creamy white lesions or plaques on the mucosal surfaces, often leading to pain, difficulty eating, and reduced quality of life (Patil *et al.*, 2018). Oral candidiasis is considered an AIDS-defining illness, particularly in individuals whose CD4+ counts fall below 200 cells/ $\mu$ L, making it an important marker for clinicians to monitor (Smith *et al.*, 2022).

Oral health problems in HIV-positive individuals are not only common but can also serve as indicators of broader health challenges. Oral candidiasis, for example, can impair basic functions such as eating and swallowing, which in turn can worsen nutritional intake and overall health

(Kehinde and Lawal, 2020). Additionally, the presence of this condition can further stigmatize individuals living with HIV, affecting their mental health and possibly hindering adherence to antiretroviral therapy (ART) (Patel *et al.*, 2019).

Despite the global rollout of ART, which has been instrumental in reducing the incidence of opportunistic infections, oral candidiasis continues to be a persistent problem in patients with poor ART adherence, high viral loads, or advanced stages of the disease (Pappas *et al.*, 2018). ART can significantly restore immune function, but in areas with limited healthcare resources, such as many parts of sub-Saharan Africa, inconsistent access to ART and poor healthcare infrastructure exacerbate the challenges of managing opportunistic infections like oral candidiasis (Okoh *et al.*, 2020). In these settings, delayed diagnosis and inadequate treatment of oral infections worsen the health outcomes of HIV-positive patients.

In sub-Saharan Africa, and specifically, Nigeria, where HIV remains highly prevalent, oral candidiasis is a frequent concern due to the challenges in accessing consistent healthcare services and ART (Kehinde and Lawal, 2020). For cities like Benin City in Edo State, addressing the prevalence and impact of oral candidiasis among HIV-positive patients requires a better understanding of the local epidemiology (Olumide *et al.*, 2021). This understanding is vital for designing effective public health interventions and improving the overall well-being of affected individuals.

## **1.2 STATEMENT OF THE PROBLEM**

HIV-infected individuals are particularly vulnerable to opportunistic infections due to their compromised immune systems, with oral candidiasis being one of the most common infections affecting this population (Akpan and Morgan, 2021). Despite the widespread availability of

antiretroviral therapy (ART), oral candidiasis remains a persistent health issue, especially in resource-limited settings such as Benin City, Edo State, Nigeria.

Currently, there is a lack of recent, region-specific data on the prevalence and antifungal susceptibility pattern of oral candidiasis among HIV-positive individuals in Benin City. This absence of localized data makes it difficult for healthcare providers to diagnose, treat, and prevent the condition effectively. Additionally, important contributing factors such as ART adherence, and socio-economic conditions are not well understood, leading to delayed diagnoses and inadequate treatment plans.

### **1.3 JUSTIFICATION OF THE STUDY**

Oral candidiasis is a common infection among people with weakened immune systems, especially those living with HIV. This study is important because it focuses on how common this infection is among HIV-infected patients in Benin City as well as the current antifungal susceptibility pattern. By identifying the prevalence and risk factors, this research can help healthcare workers improve early diagnosis and treatment approaches for these patients. The findings could also support future public health policies and intervention strategies. Overall, this study aims to contribute to better care and quality of life for people with HIV in Nigeria, especially those dealing with complications like oral candidiasis.

### **1.4 AIM OF THE STUDY**

The aim of this study is to determine the prevalence and antifungal susceptibility pattern of oral candidiasis among HIV-positive patients in Benin City, Edo State, Nigeria.

## **1.5 SPECIFIC OBJECTIVES**

1. To determine the prevalence of oral candidiasis among HIV-infected patients in Benin City hospitals.
2. To determine the antifungal sensitivity patterns of *Candida* species isolated from HIV-infected patients in Benin City hospitals.
3. To examine the associations between the demographic factors (such as age, or duration of HIV infection) and the prevalence of oral candidiasis in these patients.

## **1.6 RESEARCH QUESTIONS**

1. What is the prevalence of oral candidiasis among HIV-infected patients in Benin City?
2. What are the antifungal sensitivity patterns of *Candida species* isolated from HIV-infected patients in Benin City hospitals?
3. Are there any associations between the patient's demographic factors (such as age, or duration of HIV infection) and the prevalence of oral candidiasis?

## **1.7 RESEARCH HYPOTHESIS**

**1.7.1 Null Hypothesis (H<sub>0</sub>):** There is no significant link between the prevalence of oral candidiasis and factors like age or duration of HIV infection among patients in Benin City hospitals. Additionally, the *Candida* species found in these patients do not show any significant differences in their response to antifungal treatments.

**1.7.2 Alternative Hypothesis (H<sub>1</sub>):** There is a significant link between the prevalence of oral candidiasis and factors like age or duration of HIV infection among patients in Benin City

hospitals. Additionally, the *Candida* species found in these patients show significant differences in their response to antifungal treatments.

## CHAPTER TWO

### 2.0

### LITERATURE REVIEW

#### 2.1 *CANDIDA*

*Candida* species are opportunistic pathogens that can cause infections in individuals with weakened immune systems or underlying health issues. *Candida albicans* is the species most commonly associated with oral lesions, though other species like *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. dubliniensis* have also been found in saliva samples of individuals with and without oral candidiasis. However, finding *Candida* in the oral cavity doesn't necessarily mean there's an infection, as these organisms often colonize areas like the tongue, palate, and buccal mucosa. They may also be found in subgingival plaque in adults with periodontitis. (Sardi *et al.*, 2010)

*Candida* species have several virulence factors that support their growth and survival in the oral environment. They can form biofilms by attaching to epithelial cells and co-aggregating with bacteria, and they may even invade gingival tissues. This interaction contributes to microbial colonization and may play a role in the progression of oral diseases. Additionally, *Candida* species produce enzymes like collagenases and proteinases that break down extracellular matrix proteins and immunoglobulins, adding to their ability to cause disease. (Sardi *et al.*, 2010)

#### 2.2 *Candida albicans*

##### **Description:**

*Candida albicans* is the most common type of fungus involved in human infections and is typically found naturally in places like the mouth, digestive tract, and genitals. It can take on different shapes, including a simple yeast form, elongated pseudohyphae, or true hyphae (long,

branching structures). This flexibility helps *C. albicans* thrive in various environments in the body and makes it hard to eliminate (Sardi *et al.*, 2013).

**Pathogenicity:**

*C. albicans* becomes harmful, especially in people with weakened immune systems, because it has several ways to cause infection. It attaches easily to human cells and surfaces, releases enzymes that break down tissues, and forms biofilms (thick clusters of cells that stick to surfaces). Biofilms protect *C. albicans* from the immune system and antifungal drugs, making infections more difficult to treat (Akpan and Morgan, 2002).

This species is the primary culprit in oral candidiasis, especially among HIV-positive individuals. Its hyphal form, which can penetrate mucosal tissues, leads to stubborn infections. For people with weakened immune systems, *C. albicans* often cause painful mouth lesions, making it hard to eat and talk comfortably (Sardi *et al.*, 2013; Akpan and Morgan, 2002).

### **2.3 *Candida glabrata***

**Description:**

*Candida glabrata* is an emerging cause of infection in people with weak immune systems. Unlike *C. albicans*, it mainly exists in a simple yeast form and doesn't switch to other shapes, which makes it less flexible but still a significant threat in hospitals (Rodrigues *et al.*, 2014).

**Pathogenicity:**

*C. glabrata* has a natural resistance to azole antifungals (a standard treatment for candidiasis), making infections harder to treat. It can also stick to human cells and survive within them, avoiding immune attacks, which is important for long-term survival in the host (Sanguinetti *et al.*, 2015).

Though less aggressive than *C. albicans*, *C. glabrata* has been found more frequently in patients with long-term antifungal treatment, such as HIV-positive individuals. Its resistance to drugs allows it to thrive in the oral cavity even when other *Candida* species are reduced, causing recurring infections (Sanguinetti *et al.*, 2015).

## **2.4 *Candida tropicalis***

### **Description:**

*Candida tropicalis* is often found in tropical climates and is a significant cause of infection in people with low immunity. It grows well on surfaces as biofilms, which are protective communities of cells that increase its resistance to treatment (Silva *et al.*, 2012).

### **Pathogenicity:**

This species is known for its ability to produce enzymes that break down tissues, aiding its invasion of the body. It also forms biofilms that make it more resistant to antifungal drugs, similar to *C. albicans*. This makes *C. tropicalis* particularly troublesome in immunocompromised patients, where infections may be harder to clear (Mora-Lee, *et al.*, 2023). Although less common than *C. albicans*, *C. tropicalis* appears in some cases of oral infections, especially in patients with HIV. Its biofilm production allows it to persist in the mouth, making infections harder to treat and more likely to return (Silva *et al.*, 2012).

## **2.5 *Candida krusei***

### **Description:**

*Candida krusei* is less common but significant because it's naturally resistant to fluconazole, one

of the main drugs used to treat *Candida* infections. It has a simple yeast form, which doesn't change to other shapes, limiting its invasiveness (de Almeida *et al.*, 2019).

**Pathogenicity:**

While not as virulent as *C. albicans*, *C. krusei* is often found in healthcare settings because it can form biofilms on medical devices. Biofilms protect it from treatments and allow it to spread more easily in hospitals, where patients with weakened immune systems may be at risk (Challacombe *et al.*, 2015).

In cases of oral candidiasis, *C. krusei* is less common but can show up in patients who have had extensive antifungal treatment, especially HIV-positive patients. Because of its natural drug resistance, it can be difficult to treat, sometimes requiring alternative drugs (de Almeida *et al.*, 2019).

## **2.6 *Candida parapsilosis***

**Description:**

*C. parapsilosis* is often found on human skin, especially on the hands, which makes it a common source of infections in healthcare settings. Unlike *C. albicans*, it doesn't produce hyphae, but it does form biofilms that allow it to survive on medical devices (Trofa *et al.*, 2008).

**Pathogenicity:**

This species is less virulent but can still cause infections by forming biofilms, especially on catheters and other medical equipment. This makes it more of a problem in hospitals where it can spread through contact and pose risks to patients with compromised immune systems (Weinberger *et al.*, 2014).

*C. parapsilosis* is less common in oral infections but can emerge in immunocompromised individuals, especially those exposed to prolonged antifungal treatment. It can lead to persistent infections in such cases due to its biofilm-forming ability (Trofa *et al.*, 2008; Weinberger *et al.*, 2014).

## **2.7 *Candida guilliermondii***

### **Description:**

*C. guilliermondii* is usually harmless but has been increasingly found in infections in patients with low immunity. Unlike *C. albicans*, it doesn't form hyphae, which limits its ability to spread deeply in tissues, although it can still be persistent (Muro *et al.*, 2017).

### **Pathogenicity:**

Though not as aggressive, *C. guilliermondii* can form biofilms and develop resistance to antifungal medications, which is why it is a concern for immunocompromised patients. Its ability to survive on surfaces can make infections difficult to treat in healthcare settings (Rodero *et al.*, 2014).

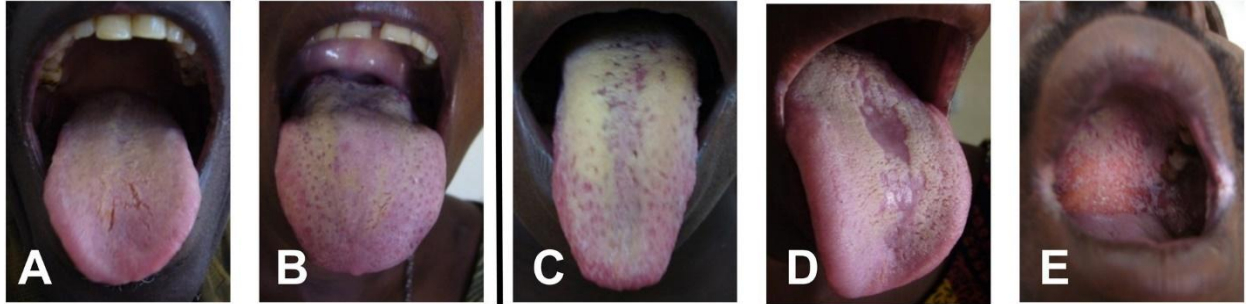
*C. guilliermondii* is rarely the primary cause of oral infections, but it can play a role in persistent oral candidiasis cases among immunocompromised individuals. Its resistance traits make it challenging to manage in these patients (Muro *et al.*, 2017; Rodero *et al.*, 2014).

## **2.8 OVERVIEW OF ORAL CANDIDIASIS**

Oral candidiasis is a common fungal infection affecting both healthy and immunocompromised individuals, particularly prevalent in early childhood and among the elderly. The infection

typically manifests as inflammation of the buccal and palatal mucosa, as well as the tongue. Factors influencing the development of oral candidiasis include immune status, oral tissue conditions, the oral environment, and various microbial elements. Clinically, it can be categorized into five types: pseudomembranous candidiasis, erythematous candidiasis, chronic atrophic candidiasis, angular cheilitis, and chronic hyperplastic candidiasis. Histopathological examination often reveals chronic inflammatory cell infiltration beneath the infected epithelium and the formation of microabscesses around *Candida* cells (Feller *et al.*, 2014).

1. **Pseudomembranous Candidiasis:** The most common form, showing white, creamy plaques on the mucosa, which can be scraped off.
2. **Erythematous Candidiasis:** Red, flat patches on the mucosa, particularly on the palate or tongue.
3. **Hyperplastic Candidiasis:** Chronic white plaques that cannot be removed by scraping.
4. **Angular Cheilitis:** Cracks and redness at the corners of the mouth, sometimes occurring alongside other types of candidiasis (Patel, 2022).



**Figure 2.1.** Representative samples of oral symptoms of the tongue and palate: **(A, B)** mild symptoms with less than 50% coverage of the tongue by whitish plaque, **(C)** >50% of tongue surface covered with whitish plaque, **(D)** median rhomboid glossitis with central atrophy, and **(E)** involvement of palate. (Taverne-Ghadwal, *et al.*, 2022).

## **2.9 OVERVIEW OF HIV/AIDS**

HIV (Human Immunodeficiency Virus) is a virus that compromises the immune system by targeting CD4 cells, which play a crucial role in immune defense. AIDS (Acquired Immune Deficiency Syndrome) is the advanced stage of HIV infection, marked by a significantly weakened immune system that leaves the body vulnerable to opportunistic infections and cancers. The first cases of HIV/AIDS were reported in the early 1980s, when clusters of rare infections, such as *Pneumocystis pneumonia* and Kaposi's sarcoma, were identified among homosexual men in the U.S. By 1983, researchers had pinpointed HIV as the virus responsible for AIDS, leading to increased public health awareness and responses to the epidemic (Ottaria *et al.*, 2018).

HIV has been a significant global health issue in recent decades, causing a chronic infection that, if untreated, progresses to AIDS. The 2015 Global AIDS Update by WHO and UNAIDS reported that 36.7 million people were living with HIV worldwide, including 1.8 million children, with 2.1 million new infections and 1.1 million deaths that year alone (World Health Organization, 2016). Initially asymptomatic, HIV gradually weakens the immune system by targeting T-helper cells, eventually leading to immunosuppression. This weakened state leaves individuals vulnerable to opportunistic infections, among which oral lesions are common and often serve as one of the earliest indicators of HIV-related immune decline (Ottaria *et al.*, 2018).

## **2.10 RELATIONSHIP BETWEEN HIV AND ORAL CANDIDIASIS**

Oral candidiasis is one of the earliest indicators of immune system decline in HIV patients. As the virus depletes CD4+ T-cells, the immune system's ability to control infections diminishes, making patients more susceptible to opportunistic pathogens like *Candida*. Oral candidiasis is one of the most frequently observed opportunistic infections in HIV-positive individuals,

especially in untreated or advanced stages of the disease (Samaranayake *et al.*, 2019). The presence of this condition is also often associated with increased morbidity, particularly if it remains untreated, as it signals a significant decline in immune function (Ranganathan *et al.*, 2020).

## **2.11 PREVALENCE OF ORAL CANDIDIASIS**

A recent extensive review of studies on oral manifestations of HIV worldwide since the start of the pandemic showed differences in how the disease progresses in low-resource countries compared to developed ones. Up until 1990, most research on this topic came from developed countries. However, between 1990 and 2000, studies were more balanced, coming equally from both developed and developing countries. From 2000 to 2017, about 86% of the studies were conducted in developing countries. This shift likely reflects the decrease in HIV-related oral issues in the Western world, leading to less accessible study material and a reduced emphasis on oral HIV research as a priority. (Tappuni, 2020).

Oral candidiasis has been the most frequently observed oral manifestation of HIV worldwide, from the start of the epidemic to today. Studies show that Africa has the highest prevalence rate of oral candidiasis at 51%, followed by Asia at 39%. In Europe and the USA, the rates are similar, averaging 28% and 30% respectively. Most studies categorized oral candidiasis into different types, with pseudomembranous being the most common, followed by erythematous, angular cheilitis, and hyperplastic candidiasis. Hyperplastic candidiasis was particularly common in African studies. (Tappuni, 2020). These findings emphasize the importance of early diagnosis and effective treatment in reducing the burden of opportunistic infections.

Different studies have reported various prevalence rates for mucocutaneous disorders in children with HIV. The prevalence rate of 53.5% in Nigeria is similar to findings from cross-sectional studies in Thailand and Chile, which reported rates of 51.6% and 56%, respectively. However, our rate is slightly lower than those reported in other studies from Africa. This difference may be influenced by factors such as the age range of patients, sample size, and antiretroviral therapy (ART) usage. For example, a study in Tanzania with an age range of 6 months to 16 years, a sample size of 374, and a prevalence of 85% showed higher rates (Katibi *et al.*, 2016). This prevalence is often linked to inadequate access to antiretroviral therapy (ART) and other healthcare resources, which can lead to increased disease susceptibility and persistence.

In Cameroon, a study found that 52.7% of HIV patients experienced oral candidiasis, particularly those with severe immunodeficiency. While *Candida albicans* remains the dominant strain, researchers also identified non-albicans species such as *C. glabrata* and *C. tropicalis*, complicating treatment options, especially in areas facing antifungal resistance (Boukeng, *et al.*, 2024).

Another significant study conducted in Ethiopia reported *Candida albicans* is one of the most commonly isolated yeasts in clinical labs from patients with HIV/AIDS. Research has shown that it can make up to 75% of the yeasts found at infection sites. In this study, the isolation rate of *C. albicans* was 61.8%, which is similar to rates reported in Mexico (58.3%) and São Paulo, Brazil (66.4%).

It's been suggested that the 'trailing growth' effect in test media can make it difficult to accurately determine MIC (Minimum Inhibitory Concentration) endpoints for some azole antifungals. Some studies recommend using spectrophotometric readings in broth microdilution

tests for a more precise MIC measurement. In our study, we used spectrophotometric readings to determine MIC endpoints.

Although several antifungal drugs are now available for treating *Candida* infections, global studies indicate that *C. albicans* strains have a relatively high potential to develop resistance. In the study, we also observed a relatively high resistance of *C. albicans* to the commonly tested antifungal drugs (Wabe, *et al.*, 2011).

These findings highlight the critical need for enhanced diagnostic practices and treatment strategies in managing oral candidiasis among HIV patients in sub-Saharan Africa, particularly considering the challenges posed by limited healthcare access and increasing antifungal resistance.

## **2.12 DIAGNOSTIC METHODS FOR ORAL CANDIDIASIS**

The diagnosis of oral candidiasis is primarily based on clinical examination and laboratory tests. Clinicians typically recognize pseudomembranous candidiasis by its characteristic white plaques, which can be wiped away to reveal erythematous mucosa underneath. Erythematous and hyperplastic forms may require laboratory confirmation, using methods such as:

1. **Direct Microscopy:** Scraping the lesion for microscopic examination to identify yeast cells or pseudohyphae.
2. **Culture:** Swabbing the affected area and culturing it to identify the specific *Candida* species.
3. **Histopathology:** For hyperplastic candidiasis, biopsy and histological examination may be required for a definitive diagnosis (Pappas *et al.*, 2019).

### 2.13 RISK FACTORS AMONG HIV PATIENTS

Several factors increase the likelihood of oral candidiasis in individuals with HIV, including:

1. **Low CD4 Count:** HIV patients with CD4 counts below 200 cells/ $\mu$ L are at higher risk due to the weakened immune response. (Erfaninejad *et al.* 2022)
2. **Non-Adherence to Antiretroviral Therapy (ART):** Inconsistent or poor adherence to ART allows HIV to progress, leading to immunosuppression and a higher risk of opportunistic infections like candidiasis. (Centers for Disease Control and Prevention [CDC], 2024)
3. **Poor Oral Hygiene:** Inadequate oral hygiene practices can foster the overgrowth of *Candida* species in the oral cavity. (Ambe *et al.* 2020)
4. **Use of Antibiotics or Steroids:** Prolonged use of these medications disrupts the natural balance of microorganisms in the mouth, promoting *Candida* proliferation. (National Institutes of Health [NIH], 2024)
5. **Smoking and Alcohol:** Both smoking and excessive alcohol consumption have been linked to an increased risk of oral candidiasis, especially in HIV-infected individuals. (Erfaninejad *et al.* 2022)

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Study Area**

The study was conducted at Central Hospital in Benin City, Edo State in Nigeria. This served as a referral for other healthcare facilities in and around the Benin metropolis.

Benin City is one of the major urban centers in Nigeria, with a diverse population comprising various ethnic groups, primarily the Edo (Bini) people. The city has a growing population, driven by both natural increase and migration from rural areas and other parts of the country. As of recent estimates, the population is over 1.5 million, with a mix of different age groups and a relatively young demographic profile.

#### **3.2 Study Population:**

The study population included HIV-positive patients receiving treatment at Central Hospital in Benin City, Edo State, Nigeria during the period of the study.

#### **3.3 Inclusion Criteria**

- Those who tested positive for HIV in the selected hospital
- Those who consented to participate in the research

#### **3.4 Exclusion Criteria**

- Those who tested negative for HIV
- Those who did not provide informed consent

### 3.5 Sample Size Determination:

The sample size was determined using a statistical formula that considered the prevalence of oral candidiasis in a similar population and the desired confidence level and margin of error.

The required sample size was determined using the formula described.

$$n = Z^2 p * q / d^2$$

Where, n = Minimum required sample size

z = Standard Normal deviation (1.96)

p = Prevalence rate from the previous study (65.3%) (Adebola *et al.*, 2012)

$$q = 1 - p$$

d = Precision tolerance margin of error (0.05)

Therefore,

$$n = (1.96)^2 * 0.8 * (1-0.8) / 0.05^2 = 245.8$$

However, a total of 250 participants were recruited.

### 3.6 Materials

- **Data Collection Forms:** A structured questionnaire was developed to collect demographic information, medical history, and clinical manifestations from the participants.
- **Laboratory Testing Kits:**
  - Sterile swabs: For collecting oral samples.
  - Commercially prepared dehydrated agar was utilized and prepared following the manufacturers' instructions. These include Sabouraud Dextrose Agar (SDA) and Mueller-Hinton Yeast Agar

- Incubator: For incubating cultures at appropriate temperatures.
- Microscope, Slides, and Coverslips: for examining fungal morphology.
- Gram stain and reagents: Crystal violet, Lugol's iodine, Acetone, Neutral red.
- Antifungal sensitivity discs: Fluconazole (25µg), Voriconazole (1µg), Caspofungin (5µg), Nystatin (100 units), Amphotericin B (20µg), Ketoconazole (15µg),
- Distilled Water: For preparing media and reagents.
- Petri Dishes; For Culturing samples.
- Personal Protective Equipment (PPE): Gloves, Lab coats, Masks, etc.

### **3.7 Sampling Technique**

The study utilized convenience sampling, a non-probability technique used to select accessible participants. Eligible individuals who met the inclusion criteria and were willing to participate in the research were recruited from the selected hospitals in Benin City, Edo State, Nigeria.

### **3.8 Demographic Data Recording**

Demographic information (age, gender, duration of HIV infection, antiretroviral therapy status) was recorded using a structured questionnaire.

### **3.9 Oral Specimen Collection**

A sterile cotton swab was used to collect oral specimens from each participant (swabbing from the teeth, gums, tongue, and cheeks). These samples were transported immediately to the Department of Medical Microbiology laboratory at the University of Benin Teaching Hospital in Benin City, Edo State Nigeria for proper scientific examination.

### **3.10 Methods**

#### **3.10.1 Microscopy Using Gram Staining Method**

Oral swab samples were examined microscopically for the presence of pus cells, budding yeast cells, and branching pseudohyphae. The Gram staining method helps identify *Candida* species and distinguish *Candida* from bacteria. The sample was smeared onto a clean grease-free glass slide, heat-fixed, and stained with Crystal violet, followed by Iodine, Acetone decolourization, and Neutral red. Under the microscope, *Candida* appears as purple oval-shaped yeast cells, often with budding. This provides a preliminary identification which is confirmed through culture and sensitivity testing.

#### **3.10.2 Culture**

Culturing is used to isolate and identify *Candida species* from clinical samples. The sample was inoculated onto Sabouraud Dextrose Agar and incubated at 37°C for 24-48 hours. Colonies appear as creamy, white, and smooth, characteristic of *Candida species*.

### **3.11 Antifungal Drug Susceptibility Test**

Susceptibility testing for the *Candida* isolates was performed using the Kirby-Bauer disk diffusion method, with interpretations made in accordance with the guidelines set by the Clinical and Laboratory Standards Institute (CLSI, 2020). The antifungal agents evaluated in this testing included fluconazole, voriconazole, ketoconazole, amphotericin B, and nystatin. This approach is widely recognized for its effectiveness in determining the resistance patterns of fungal pathogens and aids in selecting appropriate therapeutic options for managing infections

A standardized inoculum of the fungal isolate was spread on an agar plate. Then filter paper disks impregnated with antifungal agents were placed on the surface of the agar. The plates were

incubated for 24-48 hours at 35-37°C and the zones of inhibition around the disks were measured to determine susceptibility or resistance (Ekwealor *et al.*, 2023).

### **3.12 Data Analysis**

Data was analyzed using SPSS (Statistical Package for Social Sciences) and relevant statistical results were obtained. Results were presented as mean  $\pm$  standard error of mean (mean $\pm$ SEM). Difference in mean were considered significant at 95% confidence level (Statistical significance will be set at  $p < 0.05$ ).

## CHAPTER FOUR

### 4.0 RESULTS

The sociodemographic characteristics of the participants reveal that most of the participants were females (72.8%). The population of participants above 50 years of age ranks highest in the age group (26%). Also, there was a high record of participants who were into business transactions (42%), followed by those who were self-employed (26%). Those who have attained up to tertiary educational level were fewer (16.4%). Also, those who are still single in marital status were few (24.8%). (Table 4.1)

Out of 250 participants that were screened for the exercise, 76 had oral candidiasis making up thirty percent (30%) (Figure 4.1), indicating a significant burden of the condition within this population.

Age was found to have a significant association with the occurrence of oral candidiasis ( $\chi^2 = 226.799$ ,  $p < 0.001$ , Table 4.1). The highest prevalence (30.3%) was observed in patients above 50 years old (Table 4.3), likely due to age-related immune decline and other comorbidities.

Gender did not show a statistically significant relationship with oral candidiasis ( $\chi^2 = 2.084$ ,  $p = 0.149$ , Table 4.4). Nevertheless, a slightly higher prevalence was noted in females (33%) compared to males (23.5%).

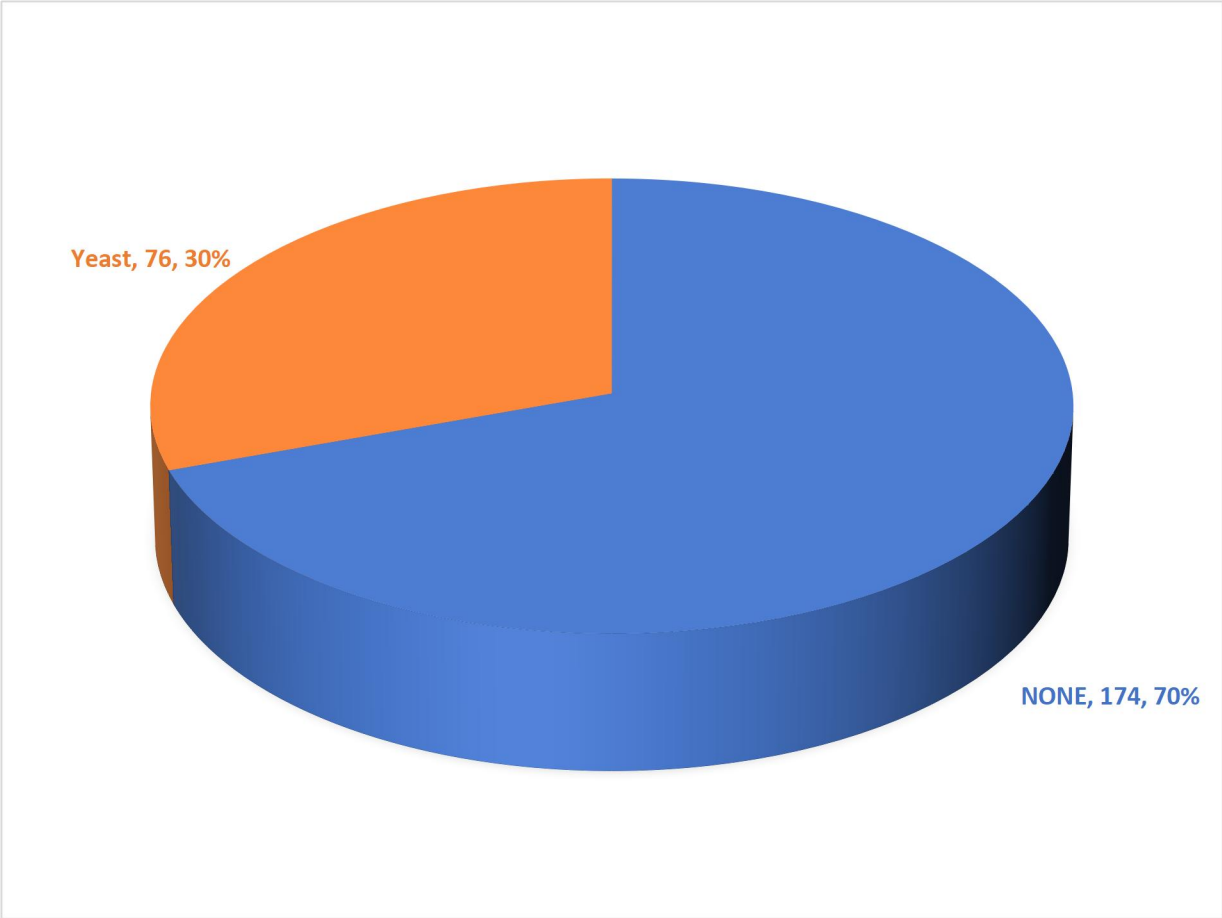
Other sociodemographic factors, including occupation, education level, and marital status, were analyzed (Tables 4.5–4.7). None of these factors showed a significant association with oral candidiasis.

The study also examined the relationship between clinical factors and the prevalence of oral candidiasis. The duration of HIV infection and ART use did not have a statistically significant impact on the prevalence of oral candidiasis ( $\chi^2 = 1.507$ ,  $p = 0.471$ , Table 4.8).

Antifungal sensitivity testing revealed the resistance and sensitivity rates of different antifungal medications. Amphotericin B (AMB) and caspofungin (CAS) had resistance rates of 30.4% and 29.2%, respectively. Ketoconazole (KTC) showed a resistance rate of 24%, while fluconazole (FLU) exhibited a resistance rate of 19.6% and a sensitivity rate of 10.8%. Nystatin (NY) had the highest sensitivity rate at 26%, followed by voriconazole (VOR) with a sensitivity rate of 11.2%. The highest resistance was observed for amphotericin B at the rate of 30.4% and caspofungin at the rate of 29.2%, while nystatin had the highest sensitivity at the rate of 26%. (Table 4.9)

**Table 4.1: Sociodemographic characteristics of participants**

<b>Variables</b>	<b>Attributes</b>	<b>Frequency</b>	<b>Percentage</b>	<b>P-value</b>
Age	<20	22	(8.8)	<0.001
	>50	66	(26.4)	
	20-25	12	(4.8)	
	26-30	15	(6.0)	
	31-35	27	(10.8)	
	36-40	43	(17.2)	
	41-45	27	(10.8)	
	46-50	38	(15.2)	
Gender	Female	182	(72.8)	0.229
	Male	68	(27.2)	
Occupation	Business	106	(42.4)	<0.001
	Civil servant	0	(0.0)	
	Employed	40	(16.0)	
	None	22	(8.8)	
	Skill	65	(26.0)	
Education	Student	17	(6.8)	<0.001
	None	32	(12.8)	
	Primary	46	(18.4)	
	Secondary	131	(52.4)	
Marital status	Tertiary	41	(16.4)	<0.001
	Divorced	19	(7.6)	
	Married	115	(46.0)	
	Single	62	(24.8)	
	Widowed	54	(21.6)	



**Figure 4.1: Prevalence of yeast**

**Table 4.2: Clinical Characteristics of Subjects**

<b>Attributes</b>	<b>Frequency</b>	<b>Percentage</b>
<b>HIV</b>		
<1	36	14.4
1 – 5	69	27.6
>5	145	58
<b>ART</b>		
<1	36	14.4
1 – 5	69	27.6
>5	145	58
No	0	0
<b>Medications</b>		
Arthritis	01	0.4
B.P.	06	2.4
B.P., TYPHOID	1	0.4
Depression	1	0.4
Diabetes	7	2.8
Fibroid	1	0.4
Malaria	1	0.4
No	220	88
Pain	1	0.4
Pregnancy	4	1.6
Tooth gum ache	0	0
Ulcer	7	2.8
<b>Blood. Transfusion</b>		
No	214	85.6
Yes	36	14.4
<b>Brush</b>		
Once	187	74.8
Twice	63	25.2
<b>O.C.</b>		
No	250	100
<b>Needle</b>		
No	247	98.8
Yes	3	1.2
<b>Sex</b>		
No	4	1.6
Yes	246	98.4
<b>Alcohol /smoking.</b>		
No	184	73.6
Yes	66	26.4

**KEY: BP=Blood pressure; O.C.=Oral candidiasis already diagnosed**

**Table 4.3: Age Distribution of Oral Candidiasis among HIV Positive Patients in Benin**

<b>Age</b>	<b>No Growth</b>	<b>Growth(%)</b>	<b>P-value</b>
<20	16(72.7)	6(27.3)	0.935
>50	46(69.7)	20(30.3)	
20-25	8(66.7)	4(33.3)	
26-30	10(66.7)	5(33.3)	
31-35	16(59.3)	11(40.7)	
36-40	30(69.8)	13(30.2)	
41-45	19(70.4)	8(29.6)	
46-50	29(76.3)	9(23.7)	

**Table 4.4: Gender Distribution of Oral Candidiasis among HIV Positive Patients in Benin**

	<b>No Growth</b>	<b>Growth(%)</b>	<b>P-value</b>	
<b>Gender</b>				
F	122(67.0)	60(33.0)	2.084	0.149
M	52(76.5)	16(23.5)		

**KEY: F=Female; M=Male**

**Table 4.5: Distribution of Oral Candidiasis based on Occupation in Patients with HIV in Benin**

<b>Occupation</b>	<b>No Growth</b>	<b>Growth(%)</b>	<b>P-value</b>	
Business	76(71.7)	30(28.3)	3.227	0.521
Civil servant	0(0.0)	0(0.0)		
Employed	29(72.5)	11(27.5)		
None	12(54.5)	10(45.5)		
Skill	44(67.7)	21(32.3)		
Student	13(76.5)	4(23.5)		

**Table 4.6: Distribution of Oral Candidiasis based on Education in Patients with HIV in Benin**

	<b>No Growth</b>	<b>Growth(%)</b>	<b>P-value</b>	
<b>Education</b>				
None	22(68.8)	10(31.3)	0.037	0.998
Primary	32(69.6)	14(30.4)		
Secondary	91(69.5)	40(30.5)		
Tertiary	29(70.7)	12(29.3)		

**Table 4.7: Distribution of Oral Candidiasis based on Marital Status in Patients with HIV in Benin**

	<b>No Growth</b>	<b>Growth(%)</b>	<b>P-value</b>	
<b>Marital status</b>				
Divorced	15(78.9)	4(21.1)	1.205	0.752
Married	81(70.4)	34(29.6)		
Single	41(66.1)	21(33.9)		
Widowed	37(68.5)	17(31.5)		

**Table 4.8: Association between clinical parameters and prevalence of yeast**

	No Growth	Growth(%)	P-value	
<b>HIV</b>				
<1	24(66.7)	12(33.3)	0.471	
>5	98(67.6)	47(32.4)		
1-5	52(75.4)	17(24.6)		
No	0(0.0)	0(0.0)		
<b>ART</b>				
<1	24(66.7)	12(33.3)	0.471	
>5	98(67.6)	47(32.4)		
1-5	52(75.4)	17(24.6)		
No	0(0.0)	0(0.0)		
<b>Medications</b>				
Arthritis	1(100.0)	0(0.0)	0.640	
BP	5(83.3)	1(16.7)		
BP+typhoid	0(0.0)	1(100.0)		
Depression	1(100.0)	0(0.0)		
Diabetes	6(85.7)	1(14.3)		
Fibroid	1(100.0)	0(0.0)		
Malaria	0(0.0)	1(100.0)		
No	151(68.6)	69(31.4)		
Pain	1(100.0)	0(0.0)		
Pregnancy	3(75.0)	1(25.0)		
Tooth gum ache	0(0.0)	0(0.0)		
Ulcer	5(71.4)	2(28.6)		
<b>Blood. Transfusion</b>				
No	148(69.2)	66(30.8)		0.712
Yes	26(72.2)	10(27.8)		
<b>Brush</b>				
Once	130(69.5)	57(30.5)	0.962	
Twice	44(69.8)	19(30.2)		
<b>O.C.</b>				
No	174(69.6)	76(30.4)		
<b>Needle</b>				
No	171(69.2)	76(30.8)	0.249	
Yes	3(100.0)	0(0.0)		
<b>Sex</b>				
No	2(50.0)	2(50.0)	0.390	
Yes	172(69.9)	74(30.1)		
<b>Alcohol/Smoking.</b>				
No	127(69.0)	57(31.0)	0.740	
Yes	47(71.2)	19(28.8)		

**KEY: BP=Blood pressure; O.C.=Oral candidiasis already diagnosed**

**Table 4.9: Antibiotics sensitivity and resistivity profile**

<b>Variables</b>	<b>Attributes</b>	<b>Frequency</b>	<b>Percentage</b>
AMB	R	76	30.4
CAS	R	73	29.2
	S	3	1.2
KTC	R	60	24
	S	16	6.4
VOR	R	48	19.2
	S	28	11.2
NY	R	11	4.4
	S	65	26
FLU	R	49	19.6
	S	27	10.8

**KEY: AMB=Amphotericin B; CAS=Caspofungin; KTC=Ketoconazole; VOR=Voriconazole; NY=Nystatin; FLU=Fluconazole.  
R=Resistant; S=Sensitive**

## CHAPTER FIVE

### 5.0 DISCUSSION

Oral candidiasis is one of the most frequently observed fungal infections in people living with HIV. Studies show that around 90% of HIV patients develop one form or another of oral candidiasis at different stages of AIDS. *Candida albicans* is the most commonly identified species linked to oral lesions in these patients. (Mahajan., *et al*, 2015.) It may serve also as an early indicator in individuals living with HIV/AIDS. (Saravani, *et al.*, 2017.; Suryana,, *et al*, 2020.; Ekwealor, *et al*, 2023.)

The study found that 30% of HIV-infected patients had oral candidiasis. This result is similar to other studies from Bida, Niger State, which reported a prevalence rate of 29.8% (Emumwen, *et al* 2017.).

Higher prevalence rates of 80% and 79.4% have been reported in Côte d'Ivoire (Kwamin, *et al*, 1995) and Ghana (Konate, *et al*, 2017), respectively. In Nigeria, a higher prevalence rate of 65.3% was reported in Anambra State (Ekwealor, *et al*, 2023.). However, other studies have found lower prevalence rates, with a prevalence rate of 12.5% in Abakaliki (Okonkwo. 2013) and as low as 9.7% reported in Jos, Nigeria. (Lar., *et al*, 2012). It is reported that these different rates could be due to the different geographical locations and different times of the studies. (Ekwealor, *et al*, 2023.)

Sociodemographic factors like gender, occupation, education level, and marital status were analyzed. Age was significantly associated with oral candidiasis, with the highest rate in patients above 50 years old. This aligns with previous research showing that older individuals are more at risk due to weaker immune systems and other health issues. (Goncalves, *et al*, 2013)

Although gender did not show a significant link to oral candidiasis, slightly more females (33%) were affected compared to males (23.5%). Other studies have shown mixed results, suggesting that factors like hormonal differences or access to healthcare may influence outcomes. (Ekwealor, *et al*, 2023.; Suryana, *et al*, 2020). It was also reported that it could be as a result of females being more predisposed to HIV/AIDS than males. (Moges, B., Bitew, A., and Shewaamare., 2016.)

Among the participants, 58% had been on ART for more than five years. Despite this, oral candidiasis remained common, which shows that ART alone might not prevent the condition. Studies suggest that while ART helps restore immune function, it may not completely stop infections, especially in patients with poor drug adherence or advanced disease. (Cauda *et al.*, 1999; Yang *et al.*, 2006).

The study found varying resistance levels to antifungal medications. Amphotericin B (AMB) had a resistance rate of 30.4% being the highest resistance rate recorded, followed by Caspofungin (CAS) which had resistance rates of 29.2%. Ketoconazole (KTC) showed a resistance rate of 24%, while fluconazole (FLU) exhibited a resistance rate of 19.6%. The resistance to fluconazole is particularly concerning since it is commonly used to treat oral candidiasis and even recommended as first choice drug due to its low toxicity, availability, e.t.c., however, it is very much available and accessible in Nigeria which could encourage self-medication and thus, suspected to be the reason for the increasing resistance. Other studies have also noted rising resistance to fluconazole among HIV patients, which underscores the need for regular antifungal. (Kuffman, 1996.; Moges., *et al.*, 2016; Aboualigalehdri, 2020.)

Nystatin however had the highest sensitivity at the rate of 26% corresponding to similar findings (Moges., *et al.*, 2016; Ekwealor, *et al*, 2023.: Hodiwala, 2021).

## **5.1 CONCLUSION**

The study highlights a high prevalence of 30% of oral candidiasis among HIV-infected patients, with age being a significant sociodemographic factor. Interestingly, other sociodemographic factors and some clinical factors such as occupation, education level, and marital status, medications taken for underlying conditions, frequency of daily tooth brush, smoking and drinking alcohol, duration of HIV infection and administration of ART did not significantly affect the occurrence of oral candidiasis among the study population. Although ART use is widespread, oral candidiasis remains prevalent, suggesting the need for continuous monitoring and management of opportunistic infections. Additionally, the observed antifungal resistance patterns emphasize the importance of personalized antifungal therapy based on sensitivity testing.

## **5.2 LIMITATIONS**

- There are a few limitations in this study. The sample size may not represent all HIV-infected patients in Benin City.
- Also, due to financial constraints, CHROMagar was not used, which would have aided in speciating the isolates, as well as the cost of CD4+ count was also beyond budget.
- Additionally, since the study was cross-sectional, it's hard to conclude cause and effect.
- The study also didn't consider other factors like adherence to ART and nutritional status, which might affect oral candidiasis.

### **5.3 RECOMMENDATIONS**

- Routine oral check-ups should be part of HIV patient care.
- Patients should avoid self-medication which leads to antifungal abuse, rather antifungal susceptibility testing should be done to guide treatment.
- Health education programs should be organized to improve ART adherence and oral hygiene.
- More research is needed to explore adherence to ART, and how nutrition and other health issues impact oral candidiasis.

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## APPENDIX I

### MEDIA AND REAGENTS PREPARATION

#### 1. Gentamicin Sabouraud Dextrose Agar (SDA)

Gentamicin-Supplemented Sabouraud Dextrose Agar (Gentamicin SDA) is prepared by dissolving 40 g of dextrose, 10 g of peptone, and 15 g of agar in 1 L of distilled water. The mixture is heated until the agar is fully dissolved, then sterilized by autoclaving at 121°C for 15 minutes. After autoclaving, the medium is allowed to cool to around 45°C, at which point 0.04 g of gentamicin is aseptically added to inhibit bacterial growth. The medium is then poured into sterile petri dishes and allowed to solidify. Prepared plates are stored at 4–8°C until required for use in fungal culture.

#### 2. Mueller Hinton Yeast Agar (MHY)

Mueller Hinton Yeast Agar (MHYA) is used for antifungal susceptibility testing, especially for *Candida* species. It is prepared by dissolving 38 g of Mueller Hinton Agar base and 5 g of yeast extract in 1 L of distilled water. The mixture is heated until completely dissolved and then autoclaved at 121°C for 15 minutes to sterilize. After sterilization, the medium is allowed to cool to about 45–50°C. Once cooled, it is poured into sterile petri dishes and allowed to solidify at room temperature. The prepared plates are stored in an inverted position at 4–8°C for future use.

## APPENDIX II

### GRAM STAINING PROCEDURE

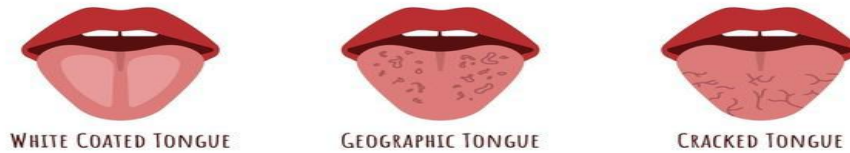
Gram staining is a vital microbiological technique used to differentiate bacterial species based on the properties of their cell walls. The procedure, developed by Hans Christian Gram in 1884, classifies bacteria into two groups: Gram-positive and Gram-negative. This distinction helps in identifying bacteria and determining appropriate treatment methods.

The procedure begins by preparing a bacterial smear on a glass slide, followed by heat fixation to adhere the bacteria to the slide. The slide is then stained with **crystal violet**, a primary stain, which imparts a purple color to all cells. After a brief incubation, the slide is washed with water and treated with **iodine solution**, which forms a complex with the crystal violet, trapping it inside the bacterial cell.

Next, the slide is subjected to a decolorization step using **ethanol or acetone**, which removes the crystal violet from Gram-negative bacteria but leaves it intact in Gram-positive bacteria. The slide is then stained with **safranin**, a counterstain, which colors the Gram-negative bacteria pink or red.

Finally, the slide is examined under a microscope. Gram-positive bacteria appear purple, while Gram-negative bacteria appear pink. This simple yet effective technique is crucial in bacterial identification and guides treatment decisions, particularly in the choice of antibiotics.

## APPENDIX III



### INFORMED CONSENT AND QUESTIONNAIRE

#### INFORMED CONSENT

Dear Sir/Ma

#### **Permission To Include You In A Research**

Title of Research: **Prevalence of Oral Candidiasis in HIV-Positive Patients in University of Benin Teaching Hospital, Benin-city in Nigeria.**

**Investigators:** 1. **DR. (MRS) I. M. MOSES-OTUTU**

Medical Microbiology, department of Medical Laboratory Science, University of Benin, Benin city.

2. **ADEBOLA SAMUEL ATURUKA**

Department of Medical Laboratory Science, University of Benin, Benin city.

**Institution address:** Department of Medical Laboratory Science,  
School of Basic Medical sciences,  
College of Medical Sciences,  
University of Benin,  
P.M.B. 1154  
Benin City, Edo State.

**Phone Number:** 08164617123

**Email:** [adebolaaturuka@gmail.com](mailto:adebolaaturuka@gmail.com)

**Proposed Duration of Research:** 3 Months

**Financial Sponsor:** Self-sponsored

**Conflict of Interest:** We declare that there is no conflict of interest

**Purpose of the Research:**

The purpose of this study is to determine the prevalence of oral candidiasis among HIV-infected patients in the University of Benin Teaching Hospital, Benin City, Nigeria

**Procedures:**

If you agree to participate in this study, you will be asked to:

- Answer a questionnaire about your demographic information, health history, and oral health.
- Undergo a clinical examination of your oral cavity by a qualified healthcare professional using disposable oral kit.

**Risks:** There are no risks to participants.

**Compensation:** Participants will neither be compensated financially nor coerced to participate.

**Confidentiality:**

All information collected in this study will be kept strictly confidential. Your identity will not be disclosed in any reports or publications resulting from this research in conferences, seminars or lectures.

**Participant’s Statement of Consent:**

I have read and understood the information provided above. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my medical care or legal rights being affected. I agree to take part in this study.

Participant’s Name: \_\_\_\_\_ Witness’s Name (If any): \_\_\_\_\_

Signature: \_\_\_\_\_ Witness’s Signature: \_\_\_\_\_

Date: \_\_\_\_\_ Date: \_\_\_\_\_

**PLEASE, KEEP A COPY OF THE SIGNED INFORMED CONSENT**

***For Official Use Only***

*Edo State Ministry of Health Approval Number* \_\_\_\_\_

*Commencement Date of the Research(dd/mm/yyyy)* \_\_\_\_\_

**QUESTIONNAIRE**

**RESEARCH STUDY: PREVALENCE OF ORAL CANDIDIASIS IN HIV-POSITIVE PATIENTS IN UNIVERSITY OF BENIN TEACHING HOSPITAL, BENIN-CITY IN NIGERIA.**

The researcher is a Bachelor of Medical Laboratory Science student from the Department of Medical Laboratory Science, University of Benin, Benin city, conducting research in Edo state.

**Please kindly ANSWER ALL questions in this section sincerely (*Your information will be kept strictly confidential*). Your cooperation and support is deeply appreciated.**

**Please do not write personal details not asked (e.g. Name, Phone number e.t.c.)**

**Demographic Information** (Please tick the data applicable to you)

1. Age: 15-25[  ]; 26-35[  ]; 36-45[  ]; 46-55[  ]; 56-65[  ]; 66-75[  ]; above 75[  ]
2. Gender: Male[  ]; Female[  ]
3. Educational Level: Primary[  ]; Secondary[  ]; Tertiary[  ]; None[  ]
4. Tribe: Edo[  ]; Yoruba[  ]; Hausa[  ]; Igbo[  ]; Others[  ]
5. Employment Status: Employed[  ]; Self Employed[  ]; Unemployed [  ]
6. Religion: Christian[  ]; Muslim[  ]; Traditionalist[  ]; Others[specify]\_\_\_\_\_

**Health Information:**

1. How long ago were you diagnosed with HIV? 0-3months[  ]; 3-6months[  ]; above 6months[  ]
2. Are you currently on antiretroviral therapy (ART)? Yes[  ]; No[  ]  
- If Yes, Have you been consistent with your doctor's appointments? Yes[  ]; No[  ]
3. Do you have any other medical conditions? Yes[  ]; No[  ]  
- If yes, please specify: \_\_\_\_\_
4. Do you smoke or take Alcohol? Yes[  ]; No[  ]

**Oral Health Information:**

1. How often do you brush daily? Once[  ]; Twice[  ]; After meals[  ]; Not Daily[  ]
2. Which of these do you use? *Tick all that apply*  
- Dental floss or interdental brushes[  ]  
- Mouthwash[  ]  
- Braces or Dentures[  ]
3. Do you experience any of the following symptoms? *Tick all that apply*  
- White patches in the mouth[  ]  
- Redness or soreness[  ]  
- Cracking at the corners of the mouth[  ]  
- Difficulty swallowing[  ]  
- Burning sensation in the mouth or throat[  ]
4. Have you been diagnosed with oral candidiasis before? Yes[  ]; No[  ]

**THANKS FOR YOUR COOPERATION.**

**APPENDIX IV**  
**ETHICAL APPROVAL**



**EDO STATE**  
**MINISTRY OF HEALTH**

<b>PROTOCOL NUMBER</b>	HA/737/24/D/1030349 (PLEASE QUOTE IN ALL ENQUIRIES)
<b>TITLE OF RESEARCH PROPOSAL</b>	PREVALENCE OF ORAL CANDIDIASIS AMONG HIV-POSITIVE PATIENTS IN HOSPITALS IN BENIN-CITY, EDO STATE, NIGERIA
<b>PRINCIPAL INVESTIGATOR (S)</b>	ADEBOLA SAMUEL ATURUKA
<b>DATE CONSIDERED</b>	30 <sup>th</sup> OCTOBER, 2024
<b>DECISION OF THE COMMITTEE</b>	APPROVED

*THIS APPROVAL DATES 30/10/2024 TO 30/10/2025. IF THERE IS A DELAY IN STARTING THE RESEARCH, PLEASE INFORM THE HREC EDO SMoH SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY*

**REMARK:** Please kindly note that the HREC Edo SMoH seal authenticates this approval

**DR (MRS) Omonyemen B. BELLO**  
(MBBS, MPH, FPHCM) (CHAIRMAN)

**SIGNATURE & DATE.....**

*Bello*  
31/10/24

**SUPERVISOR(S) .....**

**ATTESTATION BY INVESTIGATOR(S)**

No participant accrual or activity related to this research may be conducted outside of the approval dates. All informed consent forms used in this study must carry the Edo SMoH HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the Edo SMoH HREC except in circumstances outlined in the Code. The Edo SMoH HREC reserves the right to conduct compliance visits to your research site without previous notification.

**Signature & Date.....**

**APPENDIX V**  
**EXCERPTS**





