Prevalence and Distribution of *Candida* Isolates from Oral Swab Specimens of Some Women attending a Tertiary Hospital in Awka, Anambra State, Nigeria

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ABSTRACT

*Candida* species are part of the normal microbiota in healthy individuals but can cause infections in immunocompromised individuals. This study was designed to determine the prevalence and distribution of *Candida* isolates from Oral swabs (OS) collected from 246 Human Immunodeficiency Virus (HIV) positive women. Samples were cultured on appropriate media and identified using standard techniques. Statistical analysis was done using Chi-square test (p<0.05). Prevalence of *Candida* species was 36.6%. Two species each were isolated from 5 patients. *Candida* species isolated were *C. albicans* (46; 48.4%), *C. tropicalis* (13; 13.7%), *C. dubliniensis* (11; 11.6%), *C. parapsilosis* (7; 7.4%), *C. krusei* (5; 5.3%), *C. kefyr* (5; 5.3%), *C. glabrata* (4; 4.2%) and *C. guilliermondii* (4; 4.2%). Non-albicans *Candida* (NAC) species prevalence was 51.6%. Patients with CD4+ cell counts ≤200 had the highest prevalence of *Candida* colonization (53.8%) while patients with CD4+ cell counts >500 had the least (21.4%) (p=0.000). Patients on antiretroviral therapy had a lower prevalence of *Candida* colonization (34.7%) than those not on antiretroviral therapy (57.1%) (p = 0.041). Patients on antiretroviral therapy for 11-20 years had a lower prevalence of *Candida* colonization (31.6%) than those on it for 1-10 years (37.5%). This study suggests that identification of etiological agents of oral *Candida* colonization be done to species level since more non-albicans species of *Candida* are recently isolated. HIV patients should be enlightened on the benefits that strict adherence to antiretroviral therapy has on their CD4+ cell count and their overall health including reduced possibility of oral candidiasis.

1. **INTRODUCTION**

*Candida* species are commensals in healthy humans but in immunocompromised individuals, they can cause infections (Sardi *et al.*, 2013), which makes them opportunistic pathogens. They form part of the normal microbiota in mucosal oral cavity, gastrointestinal tract and vagina of an individual (Shao *et al.*, 2007). However, they are capable of causing various clinical manifestations ranging from mucocutaneous overgrowth to bloodstream infections (Eggimann *et al.*, 2003). Oral candidiasis is one of the opportunistic fungal infections that can occur in about 90% of HIV-infected individuals in the course of their illness (Wadhwa *et al.*, 2007). The
presence of Candida in the oral cavities of HIV/AIDS patients is an indicator of a subsequent development of oral candidiasis (Nweze and Ogbonna, 2011).

The prevalence of oral Candida colonization differs widely depending on age, location and immune status of the population studied (Owotade et al., 2013). It has been reported to be higher in HIV-positive individuals than their HIV-negative counterparts (Cerqueira et al., 2010), and highest among HIV patients with CD4+ cell counts less than or equal to 200 and lowest among patients with CD4+ cell counts greater than 500 (Maurya et al., 2013). Prevalence of oral Candida colonization was observed to be lower in patients on antiretroviral therapy than in patients not yet on antiretroviral therapy (Owotade et al., 2013; Cerqueira et al., 2010). Although Candida albicans remains the dominant species in oral Candida carriage of HIV-infected patients, non-albicans Candida species such as C. tropicalis, C. parapsilosis, C. guilliermondii, C. lusitaniae, C. krusei, C. kefyr and C. glabrata have also been increasingly isolated (Nweze and Ogbonna, 2011; Mushi et al., 2016).

Not very much is known about the prevalence and species distribution of different Candida species colonizing the oral cavity of HIV positive women in Nigeria especially the Southeastern region where the burden of HIV/AIDS is high. The aim of this study was to determine the prevalence and species distribution of Candida isolates from oral cavity of women attending Chukwuemeka Odumegwu Ojukwu University Teaching hospital Awka Anambra State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), a tertiary health institution located at Awka the state capital of Anambra State, Nigeria which serves as a referral centre for Anambra State and neighbouring areas. Specimen analysis took place in Microbiology Laboratory at Nnamdi Azikiwe University, Awka.

2.2 Study Design

A descriptive cross-sectional study was conducted from January to March, 2016 and a systematic random sampling technique was used.

2.3 Ethical Clearance

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by COOUTH Ethics Committee (COOUTH/AA/VOL.I.009). Written informed consent was obtained from each study participant after adequate explanation of the objectives of the study.

2.4 Inclusion and Exclusion Criteria

Consenting HIV-positive female attendees aged 16 to 65 years with or without symptoms of oral candidiasis (white patches on dorsum of tongue and buccal cavity) were recruited into the study. Exclusion criteria involved all male attendees and female attendees aged below 16 years or above 65 years. Women who had taken antifungal drugs 3 weeks before the study were verbally confirmed and excluded from the study. The participants were not paid.

2.5 Sample Collection

Oral swab specimens were collected with sterile swab sticks from 246 Human Immunodeficiency Virus (HIV) positive women attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Amaku-Awka, Anambra State, Nigeria. Structured questionnaires were administered to the patients after written consent was obtained from them. Specimens were promptly transported to Microbiology Laboratory, Nnamdi Azikiwe University, Awka for analysis.

2.6 Culture of Oral Swab (OS) Specimens

The specimens were aseptically inoculated on sterile Sabouraud dextrose agar (TM Media, Delhi, India) plates supplemented with Chloramphenicol (50μg/ml) and incubated aerobically at room temperature for 24-48hours as described by Anup et al. (2015). After the incubation period, streaking techniques were used to aseptically subculture representative discrete colonies on sterile Sabouraud dextrose agar plates to obtain pure cultures. Sterile
Sabouraud dextrose agar slants in Bijou bottles were then used to store the pure cultures at 4°C for further use. Sterile SDA plates onto which sterile swabs were streaked and incubated under the same conditions served as negative controls while a sterile SDA plate inoculated with a known positive sample obtained from a patient with oral candidiasis served as positive control. All culture media were sterilized with an autoclave (YX-400B, Shanghai, China) at 121°C for 15 minutes, while Petri dishes and test tubes were sterilized with a dry heat oven (DHG-9053A, Shanghai, China) at 160°C for 2 hours (De Broeck, 2012). The reagents used for analysis of isolates were of analytical grade.

2.7 Characterization and Identification of Isolates

The isolates were identified on the basis of colony characteristics, microscopic morphology (Anup et al., 2015), physiological and biochemical characteristics such as growth on chromogenic Candida Agar (TM Media, Delhi, India), as described by Sayyada et al. (2010), corneal agar (TM Media, Delhi, India), as described by Sayyada et al. (2010) and Anup et al. (2015), growth at elevated temperature (45°C) using an incubator (DNP-9052, Shanghai, China) as described by Pinjon et al. (1998), germ tube test (Larone, 2011), sugar fermentation test (Isu and Onyeagba, 2002) and also by comparing with photomicrographs in the colour Atlas of pathogenic fungi by Frey et al. (1979).

2.8 Statistical Analysis

The Chi-Square Test was used to test the occurrence of Candida species and comparisons of proportions between groups. Statistical Package for Social Sciences (SPSS) software for windows, version 21.0 was used for the analysis. The level of significance was set at \( p < 0.05 \).

3. RESULTS

3.1 Prevalence of Candida Colonization in HIV-positive Women

Of the 246 Oral Swabs (OS) cultured from 246 HIV-positive women, 90 yielded Candida species giving prevalence as 36.6% (Table 1).

Table 1: Prevalence of Candida Colonization in HIV-positive Women

<table>
<thead>
<tr>
<th>Population Characteristic</th>
<th>Number sampled (%)</th>
<th>Number positive Candida</th>
<th>Number negative Candida</th>
<th>%Prevalence of Candida</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-positive</td>
<td>246(100)</td>
<td>90</td>
<td>156</td>
<td>36.6</td>
</tr>
</tbody>
</table>

3.2 Occurrence of Candida species in Oral Swab Specimens

Two species each were isolated from 5 patients; a combination of \( C. \) albicans and \( C. \) dubliniensis was isolated from two patients, a combination of \( C. \) albicans and \( C. \) tropicalis was isolated from one patient, a combination of \( C. \) albicans and \( C. \) krusei was isolated from one patient while a combination of \( C. \) albicans and \( C. \) parapsilosis was isolated from one patient.

Single species were isolated from 85 cases. This makes the total species isolated to be 95. The percentage frequency of cases with single species was 94.4%. It was observed that 5.6% of the HIV patients were colonized with multiple species in their oral cavity. Non-albicans Candida species are on the increase in recent times. In this study, their prevalence was 51.6%.

\( Candida \) species isolated were \( C. \) albicans (46; 48.4%), \( C. \) tropicalis (13; 13.7%), \( C. \) dubliniensis (11; 11.6%), \( C. \) parapsilosis (7; 7.4%), \( C. \) krusei (5; 5.3%), \( C. \) kefyr (5; 5.3%), \( C. \) glabrata (4; 4.2%) and \( C. \) guilliermondii (4; 4.2%). \( C. \) albicans was the predominant species isolated (Table 2).

3.3 CD4 Range-Specific Prevalence of Oral Candida Colonization

The specific prevalence of oral Candida colonization in relation to CD4+ cell count showed that patients with CD4+ cell counts \( \leq 200 \) had the highest prevalence of 53.8%, followed by 44% prevalence among patients with CD4+ cell counts of 201-500. The lowest prevalence was found among patients with CD4+ cell counts greater than 500 (21.4%) as shown in Table 3. The lower the CD4+ cell count, the higher the rate of colonization by Candida species.
The effect of CD4+ cell count on prevalence of oral *Candida* colonization was observed to be statistically significant ($p=0.000$).

**Table 2: Occurrence of Candida species in Oral Swab Specimens**

<table>
<thead>
<tr>
<th>Species</th>
<th>Occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida tropicalis</em></td>
<td>13(13.7)</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>46(48.4)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>4(4.2)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>5(5.3)</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>11(11.6)</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>5(5.3)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>7(7.4)</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>4(4.2)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>95(100)</strong></td>
</tr>
</tbody>
</table>

**Table 3: CD4 Range-Specific Prevalence of Oral Candida Colonization**

<table>
<thead>
<tr>
<th>CD4 Range</th>
<th>Number Sampled (%)</th>
<th>Candida positive</th>
<th>Candida negative</th>
<th>Percentage of Candida positive women</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤200</td>
<td>39(15.9)</td>
<td>21</td>
<td>18</td>
<td>53.8</td>
</tr>
<tr>
<td>201–500</td>
<td>109(44.3)</td>
<td>48</td>
<td>61</td>
<td>44</td>
</tr>
<tr>
<td>&gt;500</td>
<td>98(39.8)</td>
<td>21</td>
<td>77</td>
<td>21.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>246(100)</strong></td>
<td><strong>90(36.6%)</strong></td>
<td><strong>156(63.4%)</strong></td>
<td></td>
</tr>
</tbody>
</table>

**3.4 Prevalence of Oral Candida species in relation to Antiretroviral Therapy**

Patients on antiretroviral therapy had a lower prevalence of oral *Candida* colonization (34.7%) than those who were not on antiretroviral therapy (57.1%) and the difference was statistically significant ($p = 0.041$) (Table 4). The results also showed that 11-20 years duration of antiretroviral therapy had a lower prevalence of oral *Candida* colonization (31.6%) while 1-10 years duration had a higher prevalence (37.5%) but the difference in prevalence in relation to antiretroviral therapy was not statistically significant (Table 4).

**Table 4: Prevalence of Oral Candida species in relation to Antiretroviral Therapy**

<table>
<thead>
<tr>
<th>Antiretroviral Therapy</th>
<th>Number Sampled (%)</th>
<th>Candida positive</th>
<th>Candida negative</th>
<th>Percentage of Candida positive women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>225(91.5)</td>
<td>78</td>
<td>147</td>
<td>34.7</td>
</tr>
<tr>
<td>No</td>
<td>21(8.5)</td>
<td>12</td>
<td>9</td>
<td>57.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>246(100)</strong></td>
<td><strong>90(36.6%)</strong></td>
<td><strong>156(63.4%)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – 10</td>
<td>208(84.6)</td>
<td>78</td>
<td>130</td>
<td>37.5</td>
</tr>
<tr>
<td>11 – 20</td>
<td>38(15.4)</td>
<td>12</td>
<td>26</td>
<td>31.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>246(100)</strong></td>
<td><strong>90(36.6%)</strong></td>
<td><strong>156(63.4%)</strong></td>
<td></td>
</tr>
</tbody>
</table>

**4. DISCUSSION**

In this study, prevalence of oral *Candida* colonization was 36.6%. This finding was far higher than 9.68% by Lar *et al.* (2012) in Jos, Nigeria and 12.5% by Okonkwo *et al.* (2013) in Abakaliki, Nigeria but was similar to 34.7% recorded by Enwuru *et al.* (2008) in Lagos, Nigeria. However, the prevalence recorded in this study was comparatively lower than 72.5% by Poonam *et al.* (2013) in India, 69.2% by Birhan *et al.* (2016) in Ethiopia and 60% by Nweze and Ogbonna (2011) in Abakaliki, Nigeria.
This study also isolated multiple species from five (5) of the HIV-positive individuals colonized with *Candida* species.; a combination of *C. albicans* and *C. dubliniensis* was isolated from two patients, a combination of *C. albicans* and *C. tropicalis* was isolated from one patient, a combination of *C. albicans* and *C. krusei* was isolated from one patient while a combination of *C. albicans* and *C. parapsilosis* was isolated from one patient. Enwuru *et al.* (2008) reported only one (1) patient with multiple species but Nweze and Ogbonna (2011) did not report any case of colonization with multiple species. Poonam *et al.* (2013) from India, and Vargas and Joly (2002) also isolated combinations of *C. albicans* and *C. dubliniensis* but only Poonam *et al.* (2013) and Birhan *et al.* (2016) from Ethiopia reported a combination of *C. albicans* and *C. krusei*, Vargas and Joly (2002) did not. Poonam *et al.* (2013) and Birhan *et al.* (2016) did not report a combination of *C. albicans* and *C. parapsilosis*. The prevalence of mixed culture may reflect a change from single to multiple *Candida* species isolates, which have been responsible for epidemiological shift in oropharyngeal candidiasis (Birhan *et al.*, 2016). It was observed that 5.6% of the HIV patients were colonized with multiple species in their oral cavity. Poonam *et al.* (2013) reported a lower percentage, 3.45% while Vargas and Joly (2002) had a slightly higher report, 6.8%.

*C. albicans* was the predominant species isolated (48.4%). This result was agreeable with 45% reported by Nweze and Ogbonna (2011), 40.5% reported by Enwuru *et al.* (2008) but was lower than 72.3% reported by Njunda *et al.* (2013) in Cameroon. Non-*albicans Candida* species are on the increase in recent times. In this study, their prevalence was 51.6%. This result is in tandem with 55% reported by Nweze and Ogbonna (2011) but is higher than 27.7% reported by Njunda *et al.* (2013). *C. tropicalis* (13, 13.7%) among the non-*albicans* species isolated had the highest frequency followed by *C. dubliniensis* (11, 11.6%). Nweze and Ogbonna (2011) had a similar report on *C. tropicalis* (22, 18.3%) but a lower frequency of *C. dubliniensis* (7.5%). The higher frequency of *C. dubliniensis* in this study may be as a result of the patients’ status, i.e. stage of HIV/AIDS infection (Binolfi *et al.*, 2005).

The specific prevalence of oral *Candida* colonization in relation to CD4+ cell count showed that patients with CD4+ cell counts ≤200 had the highest prevalence of 53.8%, followed by 44% prevalence among patients with CD4+ cell counts of 201-500. The lowest prevalence was found among patients with CD4+ cell counts greater than 500 (21.4%). The lower the CD4+ cell count, the higher the rate of colonization by *Candida* species. This finding agrees with Maurya *et al.* (2013) who reported highest prevalence of oral *Candida* colonization (58.7%) among HIV patients with CD4+ cell counts less than or equal to 200, 33.4% among those with CD4+ cell counts between 201 and 500, then 7.9% among patients with CD4+ cell counts greater than 500. Fong *et al.* (1997) also found a strong correlation between asymptomatic *Candida* colonization and CD4+ cell counts. However, Costa *et al.* (2006) found no correlation between *Candida* colonization and CD4+ cell counts.

Patients on antiretroviral therapy had a lower prevalence of oral *Candida* colonization (34.7%) than those who were not on antiretroviral therapy (57.1%). This observation agrees with Owotade *et al.* (2013) who reported 50% prevalence in patients on antiretroviral therapy and 100% prevalence in patients not yet on antiretroviral therapy. Cerqueira *et al.* (2010) had a similar finding that lack of antiretroviral therapy and Highly Active Antiretroviral Therapy increased the likelihood of yeast colonization. This may be because antiretroviral therapy helps to reduce viral load and increase CD4+ cell count and this in turn reduces oral *Candida* colonization.

This study also showed that 11-20 years duration of antiretroviral therapy had a lower prevalence of oral *Candida* colonization (31.6%) while 1-10 years duration had a higher prevalence (37.5%).

5. CONCLUSION

In HIV-positive patients, there is a shift in oral *Candida* colonization pattern from single species colonization with *Candida albicans* to multiple species colonization involving other species of *Candida*, although *C. albicans* is still being predominantly isolated.

Patients with CD4+ cell count ≤200 had the highest prevalence of oral *Candida* colonization followed by patients with CD4+ cell counts of 201-500, while the lowest prevalence was found among patients with CD4+ cell counts greater than 500, and the difference was significant statistically. Therefore, the lower the CD4+ cell count, the higher the prevalence of oral *Candida* colonization.

Patients on antiretroviral therapy had a lower prevalence of oral *Candida* colonization than those not on it, and those on the treatment for a longer period of time (11-20 years) had a lower prevalence of oral *Candida* colonization than those on it for fewer number of years (1-10 years).
This study suggests that identification of etiological agents of oral Candida colonization be done to species level since in recent periods, more non-albicans species of Candida are being isolated.

HIV positive patients should be enlightened on the benefits that strict adherence to antiretroviral therapy has on their CD4 count and their overall health over time including reduced possibility of oral candidiasis.

ACKNOWLEDGEMENTS

I thank Prof. Ikechukwu Okoli for his advice and oversight. Dr. (Mrs.) Ngozi-Joe Ikechebelu, the Medical doctor in charge of HIV clinic at the hospital, Mrs. Victoria Anyaoha, the Technologist at the Laboratory where the study was carried out and Mr. Chukwudi Egbuche for his assistance with the statistical analysis.

REFERENCES


How to cite this article


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