Predominance of non-*Candida albicans* species oral colonisation among patients on anticancer therapy: findings from a cross-sectional study in Tanzania

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**ABSTRACT**

**Objectives** This study aimed to determine the oral carriage prevalence of *Candida* species and identify factors associated with the carriage of *Candida* species among patients with cancer on treatment.

**Design** A hospital-based cross-sectional study.

**Setting** The study was conducted at a tertiary-level cancer hospital Ocean Road Cancer Institute (ORCI), Dar es Salaam, Tanzania.

**Participants** We enrolled 196 participants who consented to join the study. Oral swabs were collected from all participants and inoculated onto Sabouraud dextrose agar supplemented with 50 mg/ml gentamicin and 50 mg/ml chloramphenicol, and chromogenic agar for phenotypic identification of *Candida* species.

**Primary outcome** The study reported the high prevalence of oral carriage of *Candida* species among patients with cancer on treatment at the tertiary-level cancer hospital in Dar es Salaam, Tanzania.

**Results** A total of 196 participants were enrolled in the study. The overall oral carriage of *Candida* species was 37.8% (74/196). The prevalence was higher among patients undergoing chemotherapy and radiotherapy (44.4%) than those in monotherapy (13.3% chemotherapy, 20% radiotherapy). *Candida krusei* was the most common isolated species, 48.6% (36/74). Head and neck (adjusted OR (aOR) 15.09, 95% CI 3.05 to 74.59, p=0.00), gastrointestinal (aOR 14.14, 95% CI 2.25 to 88.63, p=0.00) malignancies and diabetes (aOR 3.18, 95% CI 1.03 to 9.77, p=0.04) were factors independently associated with oral carriage of *Candida* species.

**Conclusion** The oral carriage of *Candida* species among patients with cancer receiving treatment at ORCI is high, mainly due to *C. krusei* species. This is alarming since *C. krusei* has intrinsic resistance to fluconazole, a common antifungal agent used to manage adult fungal infections. Therefore, efforts should be put into conducting regular check-ups for such opportunistic pathogens as they can lead to subsequent infections. Furthermore, studies conducted to determine the antifungal profile of the causative agents are warranted since different causative agents might have different profiles.

**STRENGTHS AND LIMITATIONS OF THIS STUDY**

- We used chromogenic media for candida speciation, which can be adopted in other resource-limited settings to provide preliminary identification of *Candida* species for proper patient management.
- Unable to confirm species using biochemical and molecular tests.
- Failure to perform antifungal susceptibility testing for patient management.

**BACKGROUND**

Oral carriage of *Candida* species is the major predisposing factor to oral candidiasis in immune-compromised patients. Cancer is mentioned as an immune-compromising condition that accounts for significant morbidity and mortality. Globally it is estimated that 1 in every 3 persons will get cancer by the age of 75 years. The use of radiation therapy, chemotherapy and a combination of both is documented to compromise the patient’s immune status further and thus predisposes these patients to opportunistic infections like oral candidiasis. In addition, cancer therapy counteracts neutrophil function and induces neutrophil depletion, predisposing the person to fungal infections like oral candidiasis. The rate of oral candidiasis among patients with cancer is estimated to be 7%–52%.

Variation in the magnitude depends on the type of malignancy, whereby head and neck cancers have a higher prevalence, followed by haematological malignancies. Historically, *Candida albicans* species have been the most common cause of oral candidiasis; however, recently, non-*C. albicans* species are increasingly implicated as causative agents of candidiasis. The shift of species from *C. albicans* to non-*C. albicans* species can potentially cause treatment challenges, especially in...
resource-limited areas where treatment is usually empirical. In addition, studies have shown that *C. albicans* and non- *C. albicans* though closely related, differ in their antifungal susceptibility profiles. Therefore, identifying a specific causative agent can help inpatient management.

There are limited data on the predominant *Candida* species colonising patients with cancer undergoing cancer treatment in our geographical area. Therefore, we conducted the present study to determine the current prevalence of oral carriage of *Candida* species among patients with cancer receiving cancer treatment and evaluate the association between some factors and oral carriage.

**MATERIALS AND METHODS**

**Study design and settings**

A hospital-based cross-sectional study was conducted from July to August 2019 at Ocean Road Cancer Institute (ORCI), Dar es Salaam, Tanzania. ORCI is located along the Indian Ocean in Ilala district, Dar es Salaam, Tanzania. It is a public national referral hospital for cancer treatment in Tanzania. Currently, ORCI serves more than 50,000 patients, including about 28,000 patients with cancer, 10,000 cancer-screening patients and 12,000 patients with non-cancer. In addition, ORCI attends to over 15,000 clients in the outreach programmes in the Tanzania mainland regions.

**Study population, sample size and sampling procedure**

Adult patients aged 18 years and above on anticancer therapy present at the clinic or ward on the day of data collection were eligible for inclusion in the study. Participants were randomly selected, and those consenting were included in the study until the sample size was reached. Sample size estimation was done using the Kish Leslie formula for cross-sectional study (N=2pq/ε2); considering the prevalence of 15%, 95% CI and ε at 0.05, the estimated sample size was 196. To avoid underestimating candida oral carriage, we excluded patients with cancer who had taken antifungal agents in the past 4 weeks. In addition, patients with signs and symptoms of dry mouth were also excluded to avoid overestimating oral candida carriage. These included: mouth sores, thirstiness, cracks and cuts on lips, difficulty swallowing and loss of sense of taste.

**Data collection**

Data were collected by two research assistants who were medical doctors trained in the study protocol. A well-structured questionnaire was used to collect sociodemographic information such as age, sex, education status, employment status and clinical data, including the type of malignancy, type of anticancer treatment, stage of malignancy, treatment services (inpatient or outpatient) and diabetes status. The dependent variable was the detection of candida species by the phenotypic method.

**Sample collection and laboratory procedures**

Oral swabs were collected from each participant as per standard procedures. Briefly, a sterile cotton wool swab was used to collect the sample from the mouth of the patient. Swabs were not taken from oral lesions to avoid overestimating oral carriage. After collection, the samples were transported to Muhimbili University of Health and Allied Sciences and processed in a microbiology laboratory.

The oral swabs were inoculated into Sabouraud dextrose agar media supplemented with 50 mg/mL gentamicin and 50 mg/mL chloramphenicol (Oxoid, Basingstoke, UK) and chromogenic candida agar (CHROMagar Candida Oxoid). All media were incubated aerobically at 37°C for 24–48 hours for phenotypic identification of *Candida* species. *Candida* species were identified based on colour and colonial morphology on CHROMagar as per the manufacturer’s instructions. *C. albicans* isolates were further confirmed by germ tube test. Growth of colonies in less than three quadrants of the plate and absence of pseudo hyphae in Gram stain indicated oral candida carriage.

**Quality control**

All the reagents were prepared following the manufacturer’s instructions. In addition, we performed sterility and performance tests to check for the quality of the prepared media.

**Variables**

The independent variables were age, sex, education status, employment status, type of malignancy, type of anticancer treatment, stage of malignancy, treatment services (inpatient or outpatient) and diabetes status. The dependent variable was the detection of candida species by the phenotypic method.

**Statistical analysis**

We used STATA V15.1 software for statistical analysis. Continuous variables were summarised as the median and IQR, while proportions were used to describe categorical variables. Group differences were determined using Fisher’s exact test for categorical variables. Binary logistic regression was performed to identify factors associated with oral colonisation. In addition, multivariable logistic regression was performed to examine the associations between the outcome variable and independent variables after adjustment for other variables. At a 95% CI, factors with a p value <0.05 were considered statistically significant.

**Patient and public involvement**

 Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research study.

**RESULTS**

**Sociodemographic and clinical characteristics**

A total of 196 patients with cancer with a mean age of 54 years, an SD ±14.2, were enrolled in the study. Of the 196 participants, 69.9% were women and nearly half (87/196, 44.4%) had acquired primary education. The majority,
143/196 (73%) of the participants were inpatients, and about three-quarters, 151/196 (77%), received both chemotherapy and radiotherapy treatment. Head and neck cancers were the most prevalent type of malignancies 100/196 (51%), whereas only a few participants had gastrointestinal cancer, 7/196 (8.7%). Many participants were either in stage 2 (78/196, 39.8%) or stage 3 (73/196, 37.2%). Twenty-two participants (11.2%) had diabetes (table 1).

### Prevalence of oral colonisation of Candida species

All 196 cultured plates showed growth in less than three quadrants making the overall prevalence of oral colonisation of Candida species to be 37.8% (74/196). A higher carriage rate of 44.4% (67/151) was observed in patients treated with both chemotherapy and radiotherapy compared with each treatment separately; 13.3% (4/30) and 20% (3/15) for chemotherapy and radiotherapy, respectively (p=0.02). Patients with head and neck malignancies had a higher oral carriage, 54% (54/100) of Candida species, than other types of malignancies (p<0.0001). Although not statistically significant, the detection of Candida species was more prevalent among patients with diabetes than patients with non-diabetes; 54.5% (12/22) versus 35.6% (62/174) (p=0.08). There was no difference in the carriage rate of Candida species in other parameters such as age, gender, smoking habits, education level and cancer stage (table 2).

### Candida species isolated from patients with cancer

A total of 74 patients had one type of Candida spp in their oral cavity, making 74 candida isolates. Of the 74 Candida spp isolated, 61 (82.4%) were non-C. albicans. Candida krusei was the dominant species accounting for 48.6% (36/74), followed by Candida tropicalis (33.8%, 25/74) and lastly, C. albicans (17.6%, 13/74) (figure 1).

### Predictors of Candida species oral colonisation

On bivariate analysis, participants receiving both chemotherapy and radiotherapy treatment were five times more likely to have oral carriage of candida than other treatment types (crude OR (cOR) 5.18, 95% CI 1.72 to 15.58, p<0.0001). Likewise, in comparison to breast, cervical and prostate malignancies, patients with head and neck malignancies (cOR 14.09, 95% CI 3.16 to 62.83, p<0.0001) and those with gastrointestinal cancer (cOR 10.67, 95% CI 1.89 to 60.08, p=0.01) had an increased probability of having oral carriage of Candida spp (table 3).

After adjusting the effect of confounding factors on multivariable analysis, some types of malignancies remained associated with the oral carriage of Candida species among patients with cancer. Participants with head and neck malignancies were 15 more likely (adjusted OR (aOR) 15.09, 95% CI 3.05 to 74.59, p<0.0001) to have oral carriage of Candida species, while those with gastrointestinal cancer were fourteen more likely (aOR 14.14, 95% CI 2.25 to 88.63, p<0.0001) to have candidiasis as compared with those with breast, cervical and prostate malignancies.
In addition, the probability of being colonised by *Candida* species was three times higher among diabetic patients than non-diabetic patients (aOR 3.18, 95% CI 1.03 to 9.77, p=0.04) (table 3).

**DISCUSSION**

Oral candidiasis, usually preceded by colonisation, is a problem among immunocompromised patients with cancer, especially in cytotoxic therapy. In the present study, we report a prevalence of oral *Candida* species colonisation among patients with cancer at ORCI undergoing chemotherapy and/or radiotherapy to be 37.8%. Our finding is slightly higher compared with 25%, reported in Nagasaki, Japan, by Kawashita *et al*., and 30.1% reported in France by Gligorov *et al.* The high prevalence reported here might be attributed to the high number of patients with advanced cancer stages (2 and 3). In the present study, most participants were either in stage 2 (37.2%) or stage 3 (41.1%) of cancer, making them more prone to oral candida carriage. On the other hand, Al-Abeid *et al.* reported a much higher prevalence of candida colonisation, that is, 72.6% in Jordanian patients with cancer. The observed differences may be attributed to geographical location, population characteristics and sampling protocol.

The limited literature on oral candida carriage exists in the study settings; locally and in nearby geographical areas. However, the prevalence of oral candida carriage has been reported to be 10.3% among people living with HIV in Mwanza, while, that of the control group in the same study was reported to be 4.5%. Different methodological approaches might be a contributing factor for the observed difference, whereby a study conducted in Mwanza used a more sensitive test (Matrix-assisted laser desorption ionisation-time of flight mass spectrometry) for confirmation of candida isolates versus the use of CHROMagar in the present study which might have overestimated the reported prevalence. Furthermore, our study participants were on either chemotherapy and/or radiotherapy, a risk factor for oral candida colonisation, compared with the population used in Mwanza who were not in such therapy.

The role of cell-mediated host immunity (CMI) in controlling fungal infections is well known. Scientific evidence shows that cytotoxic chemotherapy and radiation used in treating malignancies compromise CMI, thus predisposing a person to fungal infections. In the current study, nearly all patients who had head and neck

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total number (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diabetes status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>174</td>
<td>88.8</td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>11.2</td>
</tr>
</tbody>
</table>

Others: leukaemia, lymphoma, liver, kaposi sarcoma.

*It also includes oropharyngeal cancer and oral squamous cell carcinoma.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total number</th>
<th>Candida colonisation, n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diabetes status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>174</td>
<td>62 (35.6)</td>
<td>0.90</td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>12 (54.5)</td>
<td></td>
</tr>
</tbody>
</table>

In bold, p value of <0.05 that indicates statistically significant association (Fisher’s exact test). Others: leukaemia, lymphoma, liver, kaposi sarcoma.

*It also includes oropharyngeal cancer and oral squamous cell carcinoma.
malignancies received radiotherapy and chemotherapy. As a result, oral colonisation was highest in this group (54.0%) among all patients. This result is comparable to the studies done by Lone et al, who found oral candidiasis was highest in patients with head and neck cancers compared with other types of malignancies.11

In the present study, a higher colonisation rate (44.4%) was seen in patients receiving chemotherapy and radiotherapy together than in patients receiving monotherapy (either chemotherapy or radiation therapy). Similar results were obtained in a study conducted by Jain et al which observed a significant increase in oral carriage of **Candida** species in patients taking both radiation and chemotherapy.1 This observation may be explained by the fact that cytotoxic drugs given during chemotheraphy cause dryness of oral mucosa facilitating infections by various pathogens, including fungi, and at the same time, radiation causes mucositis and changes in salivary glands, which leads to quantitative and qualitative changes in saliva, whereby thick saliva makes the oral environment conducive for fungal colonisation.17 Hence, taken together, these factors increase the chances of fungal colonisation.

Other researchers have identified **C. albicans** as the most common species causing oral colonisation.10 18 However, this was not the case in this study, we report **C. krusei** as the predominant species detected in our study setting. In addition, the predominance of **C. krusei** colonising patients on cancer therapy in the area where fluconazole is the main therapy is alarming considering the intrinsic resistance of **C. krusei** to fluconazole.12 We also report the detection of **C. tropicalis**, which has been associated with a high predictive value for invasive fungal infection.19

**Table 3** Bivariate and multivariate logistic regression for the factors associated with **Candida** oral carriage

<table>
<thead>
<tr>
<th>Variable</th>
<th>Detection of <strong>Candida</strong> spp, n (%)</th>
<th>Univariate cOR</th>
<th>95% CI</th>
<th>P value</th>
<th>Multivariate aOR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (p=0.2)</td>
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</tr>
<tr>
<td>Male</td>
<td>18 (30.5)</td>
<td>1.57</td>
<td>0.82 to 3.02</td>
<td>0.21</td>
<td>1.69</td>
<td>0.82 to 3.49</td>
<td>0.16</td>
</tr>
<tr>
<td>Female</td>
<td>56 (40.9)</td>
<td>Ref</td>
<td></td>
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</tr>
<tr>
<td>Treatment type (p=0.02)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>4 (13.3)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>3 (20.0)</td>
<td>1.63</td>
<td>0.31 to 8.43</td>
<td>0.56</td>
<td>1.97</td>
<td>0.31 to 12.55</td>
<td>0.47</td>
</tr>
<tr>
<td>Chemotherapy and radiotherapy</td>
<td>67 (44.4)</td>
<td>5.18</td>
<td>1.72 to 15.58</td>
<td>&lt;0.01</td>
<td>2.22</td>
<td>0.52 to 9.56</td>
<td>0.28</td>
</tr>
<tr>
<td>Type of malignancy (p=0.00)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Head and neck*</td>
<td>54 (54.0)</td>
<td>14.09</td>
<td>3.16 to 62.83</td>
<td>&lt;0.01</td>
<td>15.09</td>
<td>3.05 to 74.59</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>8 (47.1)</td>
<td>10.67</td>
<td>1.89 to 60.08</td>
<td>0.01</td>
<td>14.14</td>
<td>2.25 to 88.63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Breast, cervical and prostate</td>
<td>2 (7.7)</td>
<td>Ref</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>10 (18.9)</td>
<td>2.79</td>
<td>0.56 to 13.80</td>
<td>0.21</td>
<td>4.45</td>
<td>0.80 to 24.98</td>
<td>0.09</td>
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<tr>
<td>Diabetes status (p=0.08)</td>
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<tr>
<td>No</td>
<td>62 (35.6)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12 (54.8)</td>
<td>2.17</td>
<td>0.89 to 5.30</td>
<td>0.08</td>
<td>3.18</td>
<td>1.03 to 9.77</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*It also includes oropharyngeal cancer and oral squamous cell carcinoma.
aOR, adjusted OR (log-likelihood); cOR, crude OR (Binary logistic regression); Ref, reference association.
These results are worrisome as colonisation is a risk factor for infection, putting colonised patients at risk of subsequent infection. Therefore, the detection of non-*C. albicans* species, especially *C. krusei*, empathise the need for species identification and drug susceptibility testing of the infecting *Candida* species in patients with cancer before starting empirical therapy.

This study had limitations: we did not collect information about variables that could affect oral candida carriage. These variables include: duration of cancer treatment, prolonged use of antibiotics, history of dental caries and periodontal diseases. Furthermore, we did not perform biochemical and molecular tests to confirm further/differentiate *Candida* species. Also, antifungal susceptibility testing was not performed in the present study to show the antifungal profile among *Candida* species. Nonetheless, the study has demonstrated the contribution of non-*C. albicans* in the oral cavity of patients with cancer, potentially leading to subsequent infections that might be difficult to treat due to their intrinsic resistance to conventional antifungal agents.

**CONCLUSION**

Oral non-*Candida* species colonisation is high among patients with cancer at ORCI. Patients with head and neck malignancies are at increased risk of colonisation, a risk factor for subsequent infections. There is, therefore, a need for prompt identification of causative agents of candidiasis among patients with cancer and fungal susceptibility testing for better management of patients as resistance pattern differs between *C. albicans* and non-*C. albicans* species.

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**Contributors** UOK and DK were involved in the study’s conceptualisation and performed data collection and laboratory work. UOK, DK and AMM performed all the statistical analyses. UOK, DK, AMM, MMu and MMa were involved in drafting the manuscript. JM and MMa were involved in a critical review of the manuscript. UOK is acting as the guarantor for this article.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and was approved by Senate of Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS) (Reference no. DA.25/111/01). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** All data relevant to the study are included in the article or uploaded as supplementary information.

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