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# BMJ Open

## Predominance of non-Candida albicans species oral colonization among patients on anticancer therapy: A call for improved fungi diagnosis in Tanzania

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-070003
Article Type:	Original research
Date Submitted by the Author:	11-Nov-2022
Complete List of Authors:	Kibwana, Upendo; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Manyahi, Joel; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Kamori, Doreen; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Mushi, Martha; Catholic University of Health and Allied Sciences, Department of Microbiology and Immunology Mwandigha, Ambele M.; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Majigo, Mtebe; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology
Keywords:	MICROBIOLOGY, INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES

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5 2 **anticancer therapy: A call for improved fungi diagnosis in Tanzania**  
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## 28 ABSTRACT

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

32 **Design:** A hospital-based cross-sectional study

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

35 **Participants:** We enrolled 196 participants who consented to join the study. Oral swabs  
36 were collected from all participants and inoculated onto Sabouraud Dextrose Agar and  
37 chromogenic agar for phenotypic identification of *Candida* species.

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

## 41 Results

42 A total of 196 participants were enrolled in the study. The overall oral carriage of *Candida*  
43 species was 37.8% (74/196). The prevalence was higher among patients undergoing both  
44 chemotherapy and radiotherapy (44.4%) than those in monotherapy (13.3% chemotherapy,  
45 20% radiotherapy). *Candida krusei* was the commonest isolated species, 48.6% (36/74).  
46 Head and neck (aOR, 15.09, 95%CI 3.05-74.59, p=0.00), gastrointestinal (aOR, 14.14,  
47 95%CI 2.25-88.63, p=0.00) malignancies and diabetes (aOR=3.18, 95% CI=1.03-9.77,  
48 p=0.04) were factors independently associated with oral carriage of *Candida* species.

## 50 Conclusion

51 Oral carriage of *Candida* species among cancer patients receiving treatment at ORCI is high,  
52 mainly due to *C.krusei* species. This is alarming since *C.krusei* has intrinsic resistance to  
53 fluconazole, a common antifungal agent used to manage fungal infections in adults.  
54 Therefore, efforts should be put into conducting regular check-ups for such opportunistic

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4 55 pathogens as they can lead to subsequent infections. Furthermore, studies conducted to  
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6 56 determine the antifungal profile of the causative agents is warranted since, different  
7  
8 57 causative agents might have different profiles.

### 10 58 **Strengths and limitation of the study**

- 12 59 • This is the first study that reports the isolation of non *Candida albicans* species in the  
14 60 oral cavity among Tanzanian cancer patients receiving therapy in a national cancer  
16 61 institute.
- 18 62 • We could not perform antifungal susceptibility testing in the present study to show the  
20 63 antifungal profile among different *Candida* species for patient management.

### 23 65 **Keywords**

26 66 Oral candidiasis, Cancer patients, Chemotherapy and radiotherapy, Gastrointestinal  
28 67 malignancy, Head and neck malignancy

### 46 75 **BACKGROUND**

48 76 Oral carriage of *Candida* species is the major predisposing factor to oral candidiasis in  
50 77 immune-compromised patients(1). Cancer is mentioned as an immune-compromising  
52 78 condition that accounts for great morbidity and mortality(2). Globally it is estimated that 1 in  
54 79 every 3 persons suffers from cancer by 75 years(3). The use of radiation therapy,  
56 80 chemotherapy, and/or a combination of both is documented to compromise the patient's

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4 81 immune status further and thus predisposes these patients to opportunistic infections like oral  
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6 82 candidiasis(4–6). In addition, cancer therapy counteracts neutrophil's function and induces  
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8 83 neutrophil depletion, predisposing the person to fungal infections like oral candidiasis(7). It is  
9  
10 84 estimated that the rate of oral candidiasis among cancer patients ranges from 7 to 52%(8).

11  
12 85 Variation in the magnitude depends on the type of malignancy, whereby head and neck cancer  
13  
14 86 have a higher prevalence, followed by haematological malignancies(9–11). Historically, *C.*  
15  
16 87 *albicans* species have been the most common cause of oral candidiasis; however,  
17  
18 88 recently, non-*Candida albicans* species are increasingly implicated as causative agents of  
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20 89 candidiasis(12). The shift of species from *C. albicans* to non-*C. albicans* species can  
21  
22 90 potentially cause treatment challenges, especially in resource-limited areas where treatment is  
23  
24 91 usually empirical. In addition, studies have shown that *C. albicans* and non-*C. albicans*  
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26 92 though closely related, differ in their antifungal susceptibility profiles(7,13). Therefore  
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28 93 identifying a specific causative agent can help in patient management.

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31 94 There is limited data on the predominant *Candida* species colonizing cancer patients  
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33 95 undergoing cancer treatment in our geographical area. Therefore, we conducted the present  
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35 96 study to determine the current prevalence of oral carriage of *Candida* species among cancer  
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37 97 patients receiving cancer treatment and evaluate the association between some factors and  
38  
39 98 oral carriage.  
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## 47 100 **MATERIALS AND METHODS**

### 48 49 101 **Study design and settings**

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51 102 A hospital-based cross-sectional study was conducted from July to August 2019 at Ocean  
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53 103 Road Cancer Institute (ORCI) in Dar es Salaam, Tanzania. ORCI is located along the Indian  
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55 104 Ocean in Ilala district, Dar es Salaam, Tanzania. It is a public national referral hospital for  
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57 105 cancer treatment in Tanzania. Currently, ORCI serves more than 50,000 patients, including  
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4 106 about 28,000 cancer patients, 10,000 cancer screening patients, and 12,000 non-cancer  
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6 107 patients. In addition, ORCI attends to over 15,000 clients in the outreach programs in the  
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8 108 Tanzania mainland regions.

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

110 Adult patients aged 18 years and above on anticancer therapy present at the clinic or ward on  
111 the day of data collection were eligible for inclusion in the study. Sample size estimation was  
112 done using the Kish Leslie formula at a 95% confidence interval(14). To avoid  
113 underestimating *Candida* oral carriage, we excluded cancer patients who had taken antifungal  
114 agents in the past four weeks.

### 115 **Data collection**

116 A well-structured questionnaire was used to collect socio-demographic information such as  
117 age, sex, education status, employment status, and clinical information, including the type of  
118 malignancy, type of anticancer treatment, stage of malignancy, inpatient and outpatient  
119 services and diabetes status.

### 120 **Sample collection and laboratory procedures**

121 Oral swabs were collected from each participant as per standard procedures. Briefly, a sterile  
122 cotton wool swab was used to collect the sample from the mouth of the patient. Then, the  
123 samples were transported to Muhimbili University of Health and Allied Sciences (MUHAS)  
124 and processed in a Microbiology laboratory.

125 The oral swabs were inoculated into Sabouraud dextrose agar (SDA) media (Oxoid,  
126 Basingstoke RG24 8PW, UK) and chromogenic candida agar (CHROMagar Candida Oxoid).  
127 All media were incubated aerobically at 37°C for 24-48 hours for phenotypic identification of  
128 *Candida* species. *Candida* species were identified based on colour and colonial morphology  
129 on CHROM agar as per the manufacturer's instructions.



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7 131 **Quality control**8  
9 132 All the reagents were prepared following the manufactures instructions. In addition, we  
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11 133 performed sterility and performance tests to check for the quality of prepared media.  
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14 134 **Variables**15  
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17 135 The independent variables were, age, sex, education status, employment status, type of  
18  
19 136 malignancy, type of anticancer treatment, stage of malignancy, treatment services (inpatient  
20  
21 137 or outpatient) and diabetes status. The dependent variable was detection of candida species by  
22  
23 138 phenotypic method.  
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2526  
27 139 **Statistical analysis**28  
29 140 We used STATA version 15.1 software for statistical analysis. Continuous variables were  
30  
31 141 summarized as the median and interquartile range (IQR), while proportions were used to  
32  
33 142 describe categorical variables. Group differences were determined using Fisher's exact test  
34  
35 143 for categorical variables. Binary logistic regression was performed to identify factors  
36  
37 144 associated with oral colonization. In addition, multivariable logistic regression was performed  
38  
39 145 to examine the associations between the outcome variable and independent variables after  
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41 146 adjustment for other variables. At a 95% confidence level, factors with a *p-value* <0.05 were  
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43 147 considered statistically significant.  
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4647  
48 148 **Patient and public involvement**49  
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51 149 This study was designed to investigate the prevalence of oral candida carriage and the  
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53 150 causative agents among cancer patients to better plan infection prevention and control  
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55 151 practices, thus improving patient care. Patients were not involved in designing this research,  
56  
57 152 however, the proposed study was presented to the members of the department of  
58  
59 153 Microbiology and immunology of Muhimbili University of Health and Allied Sciences  
60

154 before the recruitment of participants began. Patients who were colonized with candida  
 155 species were notified as soon as the sample processing was complete and the final report  
 156 was communicated to the hospital management and the infection prevention and control  
 157 team of Ocean Road Cancer Institute.

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159

## 160 RESULTS

### 161 Socio-demographic and clinical characteristics

162 A total of 196 cancer patients with a mean age of 54 years, a standard deviation (SD)  $\pm$  14.2,  
 163 were enrolled in the study. Of the 196 participants, 69.9% were female, and nearly half  
 164 (87/196, 44.4%) had acquired primary education. The majority, 143/196 (73%) of the  
 165 participants, were inpatients, and about three-quarters, 151/196 (77%), received both  
 166 chemotherapy and radiotherapy treatment. Head and neck cancers were the most prevalent  
 167 type of malignancies 100/196 (51%), whereas only a few participants had gastrointestinal  
 168 cancer 7/196 (8.7%). Many participants were either in stage 2 (78/196;39.8%) or stage 3  
 169 (73/196;37.2%). Twenty-two participants ( 11.2%) had diabetes (Table 1).

170

171 **Table 1: Distribution of socio-demographic and clinical characteristics among cancer**  
 172 **patients (N = 196)**

Variable	Total number(N)	Percentage (%)
<b>Age group (Mean =54; SD<math>\pm</math> 14.2)</b>		
<54	101	51.5
>54	95	48.5
<b>Gender</b>		
Male	59	30.1
Female	137	69.9
<b>Educational level</b>		
Primary	87	44.4
Secondary and above	80	40.8
Non formal	29	14.8
<b>Smoking</b>		
No	165	84.2
Yes	31	15.8
<b>Patient care</b>		

Inpatient	143	73.0
Outpatient	53	27.0
<b>Treatment type</b>		
Chemotherapy	30	15.3
Radiotherapy	15	7.7
Chemotherapy and Radiotherapy	151	77.0
<b>Type of Malignancy</b>		
Head and neck	100	51.0
Gastrointestinal	17	8.7
Breast,cervical&prostate	26	13.3
Other	53	27.0
<b>Cancer stage</b>		
1	29	14.8
2	78	39.8
3	73	37.2
4	16	8.2
<b>Diabetes status</b>		
No	174	88.8
Yes	22	11.2

173 *Others: leukemia, lymphoma, liver, kaposi sarcoma*

174

### 175 **Prevalence of oral colonization of *Candida* species**

176 The overall prevalence of oral colonization of *Candida* species was 37.8% (74/196). A higher  
 177 carriage rate of 44.4% (67/151) was observed in patients treated with both chemotherapy and  
 178 radiotherapy compared to each treatment separately; 13.3% (4/30) and 20% (3/15) for  
 179 chemotherapy and radiotherapy respectively ( $p=0.02$ ). Patients with head and neck  
 180 malignancies had a higher oral carriage, 54% (54/100) of *Candida* species, than other types  
 181 of malignancies ( $p<0.0001$ ). Although not statistically significant, detection of *Candida*  
 182 species was more prevalent among diabetic patients than non-diabetic; 54.5 % (12/22) vs.  
 183 35.6 % (62/174) ( $p=0.08$ ). There was no difference in the carriage rate of *Candida* species in  
 184 other parameters such as age, gender, smoking habits, education level, and cancer stage  
 185 (Table 2).

186

187 **Table 2: Prevalence of oral *Candida* carriage among cancer patients by social-**  
 188 **demographic and clinical factors**

Variable	Total number	<i>Candida</i> colonization	<i>P</i> -value
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		n (%)	
<b>Overall</b>	<b>196</b>	<b>74 (37.8)</b>	
<b>Age group</b>			
<54	101	41 (40.6)	0.46
>54	95	33 (34.7)	
<b>Gender</b>			
Male	59	18 (30.5)	0.20
Female	137	56 (40.9)	
<b>Educational level</b>			
Primary	87	38 (43.7)	
Secondary and above	80	29 (36.3)	0.26
Non formal	29	7 (24.1)	
<b>Smoking</b>			
No	165	62 (35.6)	0.90
Yes	31	12 (38.7)	
<b>Patient care</b>			
Inpatient	143	52 (36.4)	0.51
Outpatient	53	22 (41.5)	
<b>Treatment type</b>			
Chemotherapy	30	4 (13.3)	
Radiotherapy	15	3 (20.0)	<b>0.02</b>
Chemotherapy and Radiotherapy	151	67 (44.4)	
<b>Type of Malignancy</b>			
Head and neck	100	54 (54.0)	
Gastrointestinal	17	8 (47.1)	
Breast, cervical& Prostate	26	2 (7.7)	<b>&lt; 0.01</b>
Other	53	10 (18.9)	
<b>Cancer stage</b>			
1	29	10 (34.5)	
2	78	29 (37.2)	0.85
3	73	30 (41.1)	
4	16	5 (31.3)	
<b>Diabetes status</b>			
No	174	62 (35.6)	0.08
Yes	22	12 (54.5)	

189 *Others: leukemia, lymphoma, liver, kaposi sarcoma; In bold p-value of less than 0.05 that indicates*  
 190 *statistically significant association (Fisher's exact test)*

191

## 192 **Candida species isolated from cancer patients**

193 A total of 74 patients had one type of *Candida* spp. in their oral cavity, making 74 candida  
 194 isolates. Of the 74 *Candida* spp isolated, 61(82.4%) were non-*C. albicans*. *Candida krusei*  
 195 was the dominant species accounting for 48.6% (36/74), followed by *Candida tropicalis*  
 196 (33.8%, 25/74) and lastly, *C. albicans* (17.6%, 13/74) (Figure 1).

197

## 198 Predictors of *Candida* species oral colonization

199 On bivariate analysis, participants receiving both chemotherapy and radiotherapy treatment  
 200 were five times more likely to have oral carriage of *Candida* than other treatment types  
 201 (cOR5.18, 95%CI 1.72-15.58,  $p<0.0001$ ). Likewise, in comparison to breast, cervical and  
 202 prostate malignancies, patients with head and neck malignancies (cOR,14.09, 95%CI 3.16-  
 203 62.83,  $p<0.0001$ ) and those with gastrointestinal cancer (cOR, 10.67, 95%CI 1.89-60.08,  
 204  $p=0.01$ ) had an increased probability of having oral carriage of *Candida spp* (Table 3).

205 After adjusting the effect of confounding factors on multivariable analysis, some types of  
 206 malignancies remained associated with the oral carriage of *Candida* species among cancer  
 207 patients. Participants with head and neck malignancies were 15 more likely (aOR, 15.09,  
 208 95%CI 3.05-74.59,  $p<0.0001$ ) to have oral carriage of *Candida* species, while those with  
 209 gastrointestinal cancer were fourteen more likely (aOR, 14.14, 95%CI 2.25-88.63,  $p<0.0001$ )  
 210 to have candidiasis compared to those with breast, cervical and prostate malignancies. In  
 211 addition, the probability of being colonized by *Candida* species was three times higher  
 212 among diabetic patients than non-diabetic patients (aOR=3.18, 95% CI=1.03-9.77,  $p=0.04$ )  
 213 (Table 3).

214

215 **Table 3: Bivariate and Multivariate logistic regression for the factors associated with**  
 216 ***Candida* oral carriage**

Variable	Detection of <i>Candida</i> spp, n (%)	Univariate cOR	95% CI	p-value	Multivariate aOR	95% CI	p-value
<b>Gender (p=0.2)</b>							
Male	18 (30.5)	Ref			Ref		
Female	56 (40.9)	1.57	0.82-3.02	0.21	1.69	0.82-3.49	0.16
<b>Treatment type (p=0.02)</b>							
Chemotherapy	4 (13.3)	Ref			Ref		
Radiotherapy	3 (20.0)	1.63	0.31-8.43	0.56	1.97	0.31-12.55	0.47
Chemotherapy and Radiotherapy	67 (44.4)	5.18	1.72-15.58	<0.01	2.22	0.52-9.56	0.28

Type of Malignancy (p=0.00)							
Head and neck	54 (54.0)	14.09	3.16-62.83	<0.01	15.09	3.05-74.59	<0.01
Gastrointestinal	8 (47.1)	10.67	1.89-60.08	0.01	14.14	2.25-88.63	<0.01
Breast, cervical & Prostate	2 (7.7)	Ref			Ref		
Other	10 (18.9)	2.79	0.56-13.80	0.21	4.45	0.80-24.98	0.09
Diabetes status (p=0.08)							
No	62 (35.6)	Ref			Ref		
Yes	12 (54.8)	2.17	0.89-5.30	0.08	3.18	1.03-9.77	0.04

217 *cOR* stands for crude odd ratio(Binary logistic regression), *aOR* stands for adjusted odd ratio (Log  
218 likelihood)and *Ref* stands for referenceassociation

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## 221 DISCUSSION

222 Oral candidiasis, which is usually preceded by colonization, is a problem among  
223 immunocompromised patients with cancer, especially in cytotoxic therapy. In the present  
224 study, we report a prevalence of oral *Candida* species colonization among cancer patients at  
225 ORCI undergoing chemotherapy and/or radiotherapy to be 37.8%. Our finding is slightly  
226 higher compared to 25%, which was reported in Nagasaki, Japan, by Kawashita, Y *et al.*,  
227 2011(15), and 30.1% reported in France by Grigorov J *et al.*,2010(16). On the other hand, Al-  
228 Abeid *et al.*, 2004 reported a much higher prevalence of *Candida* colonization, i.e., 72.6% in  
229 Jordanian cancer patients(14). The observed differences may be attributed to geographical  
230 location, population characteristics, and sampling protocol.

231 The role of cell-mediated host immunity (CMI) in controlling fungal infections is well  
232 known. Scientific evidence shows that cytotoxic chemotherapy and radiation used in treating  
233 malignancies compromises CMI, thus predisposing a person to fungal infections. In the  
234 current study, nearly all patients who had head and neck malignancy received radiotherapy  
235 and chemotherapy. Oral colonization was highest in this group (54.0%) among all patients.

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4 236 This result is comparable to the studies done by Lone M Set *al.*, who found oral candidiasis  
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6 237 was highest in head & neck cancer patients compared to other types of malignancies(11).

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8 238 In the present study higher colonization rate (44.4%) was seen in patients receiving  
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10 239 chemotherapy and radiotherapy together than in patients receiving monotherapy (either  
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12 240 chemotherapy or radiation therapy). Similar results were obtained in a study conducted by  
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14 241 Manish Jain *et al.*, 2016 which observed a significant increase in oral carriage of *Candida*  
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16 242 species in patients taking both radiation and chemotherapy(1). This observation may be  
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18 243 explained by the fact that cytotoxic drugs given during chemotherapy cause dryness of oral  
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20 244 mucosa facilitating infections by various pathogens, including fungi, and at the same time,  
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22 245 radiation causes mucositis and changes in salivary glands, which lead to quantitative and  
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24 246 qualitative changes in saliva, whereby thick saliva makes the oral environment conducive for  
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26 247 fungal colonization(17). Hence, taken together, these factors increase the chances of fungal  
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28 248 colonization.

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33 249 Other researchers have identified *Candida albicans* as the most common species causing oral  
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35 250 colonization(10,18). However, this was not the case in this study; we report *Candida krusei*  
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37 251 as the predominant species detected in our study setting. In addition, the predominance of  
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39 252 *Candida krusei* colonizing patients on cancer therapy in the area where fluconazole is the  
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41 253 main therapy is alarming. This is because *Candida krusei* has intrinsic resistance to  
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43 254 fluconazole(12). We also report the detection of *Candida tropicalis* which has been  
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45 255 associated with a high predictive value for invasive fungal infection(19).

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49 256 These results are worrisome as colonization is a risk factor for infection, putting colonized  
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51 257 patients at risk of subsequent infection. Therefore, detection of non- *C. albicans* species,  
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53 258 especially *Candida krusei*, emphasizes the need for species identification and drug  
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55 259 susceptibility testing of the infecting *Candida* species in cancer patients before starting  
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57 260 empirical therapy.

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4 261 This study had a limitation; we could not perform antifungal susceptibility testing in the  
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6 262 present study to show the antifungal profile among different *Candida* species.  
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## 8 263 **CONCLUSION**

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10 264 Oral non-*Candida* species colonization is high among cancer patients at ORCI. Patients with  
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12 265 head and neck malignancies are at high risk of colonization, a risk factor for subsequent  
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14 266 infections. There is therefore, a need for prompt identification of causative agents of  
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16 267 candidiasis among cancer patients and fungal susceptibility testing for better management of  
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18 268 patients as resistance pattern differs between *C. albicans* and non-*C. albicans* species.  
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## 24 271 **LIST OF ABBREVIATIONS**

25  
26  
27 272 **AIDS-** Acquired Immunodeficiency Syndrome, **HIV-** Human Immunodeficiency Virus,  
28

29 273 **OC-** Oral Candidiasis, **ORCI-** Ocean Road Cancer Institute  
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## 32 33 275 **DECLARATIONS**

### 34 35 276 **Ethics approval and consent to participate**

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38 277 Ethical clearance was obtained from the Senate of Research and Publications Committee of  
39  
40 278 the Muhimbili University of Health and Allied Sciences (MUHAS). Permission to conduct  
41  
42 279 the study was obtained from the ORCI administration. Before enrolling in the study, written  
43  
44 280 informed consent was obtained from each participant. Confidentiality of the study  
45  
46 281 participants was ensured using codes instead of participants' names.  
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50 282

### 51 283 **Consent for Publication**

52  
53  
54 284 Not applicable  
55

### 56 285 **Availability of data and materials**

57  
58  
59 286 All relevant data generated and analyzed during this study are included in this manuscript.  
60



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2  
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4 287

5  
6 288 **Competing Interests**

7  
8 289 The authors declare that they have no competing interests.

9  
10 290

11  
12  
13 291 **Funding**

14  
15 292 No funding was received for this study.

16  
17 293

18  
19 294 **Authors' contributions**

20 295 UK and DK were involved in the study's conceptualization and performed data collection and  
21  
22 296 laboratory work. UK, DK, and AMM performed all the statistical analyses. UK, DK, AMM,  
23  
24 297 MFM, and MM were involved in drafting the manuscript. JM and MM were involved in a  
25  
26 298 critical review of the manuscript.  
27  
28

29  
30 299

31  
32 300 **Acknowledgment**

33 301 The authors would like to acknowledge all patients who participated in this study.

34  
35 302

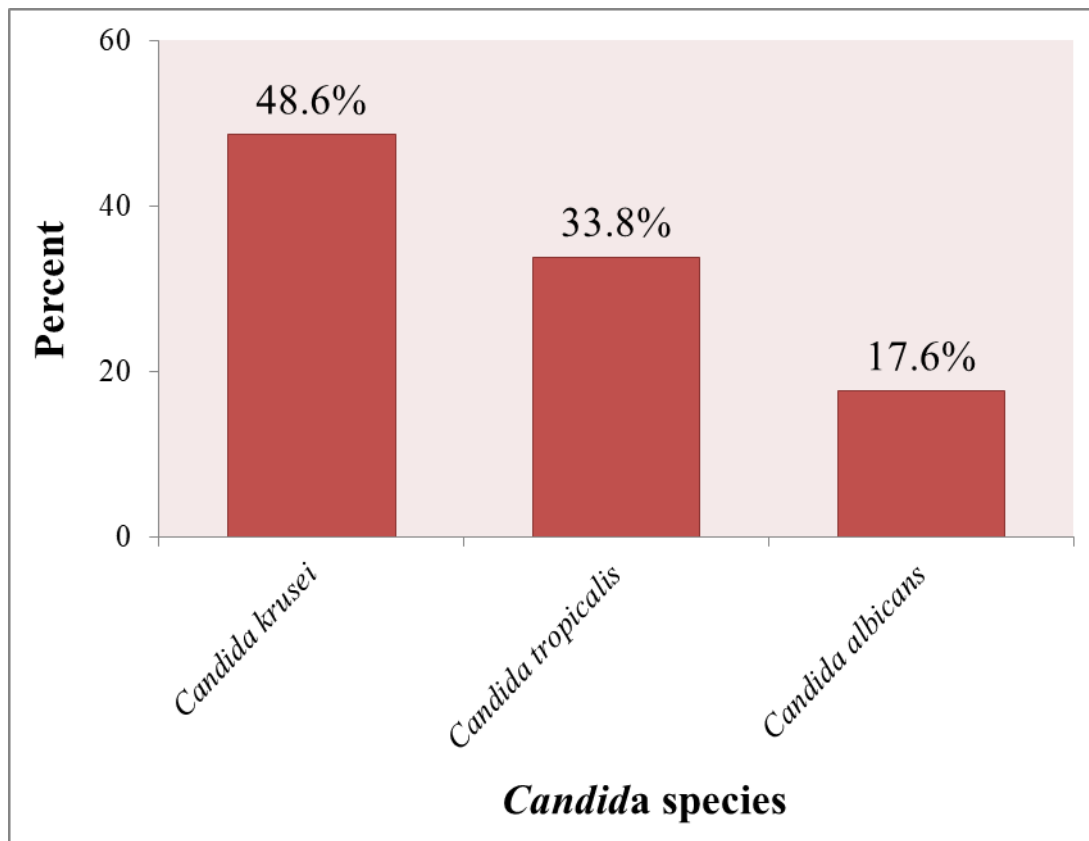
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Figure 1: Distribution of *Candida* species isolates from cancer patients



The figure illustrates the distribution of specific *Candida* species isolates that were obtained from 196 cancer patients at Ocean road Cancer Institute (ORCI).

**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cross-sectional studies**

Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Page 1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Pages 2 and 3
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	Page 5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 6
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 6
Bias	9	Describe any efforts to address potential sources of bias	Page 5
Study size	10	Explain how the study size was arrived at	Page 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pages 6 and 7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Pages 6 and 7
		(b) Describe any methods used to examine subgroups and interactions	NA
		(c) Explain how missing data were addressed	NA
		(d) If applicable, describe analytical methods taking account of sampling strategy	NA
		(e) Describe any sensitivity analyses	NA
<b>Results</b>			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pages 7 and 8
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	Pages 8 and 9
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 10
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	Page 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pages 11 and 12
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

# BMJ Open

## The predominance of non-*Candida albicans* species oral colonization among patients on anticancer therapy: Findings from a cross-sectional study in Tanzania

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-070003.R1
Article Type:	Original research
Date Submitted by the Author:	26-Jan-2023
Complete List of Authors:	Kibwana, Upendo; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Manyahi, Joel; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Kamori, Doreen; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Mushi, Martha; Catholic University of Health and Allied Sciences, Department of Microbiology and Immunology Mwandigha, Ambele M.; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Majigo, Mtebe; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology
<b>Primary Subject Heading</b>:	Diagnostics
Secondary Subject Heading:	Infectious diseases
Keywords:	MICROBIOLOGY, INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES, MYCOLOGY, Head & neck tumours < ONCOLOGY

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4 1 **The predominance of non-*Candida albicans* species oral colonization among patients on**  
5 2 **anticancer therapy: Findings from a cross-sectional study in Tanzania**  
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## 28 ABSTRACT

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

32 **Design:** A hospital-based cross-sectional study

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

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

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

## 42 Results

43 A total of 196 participants were enrolled in the study. The overall oral carriage of *Candida*  
44 species was 37.8% (74/196). The prevalence was higher among patients undergoing both  
45 chemotherapy and radiotherapy (44.4%) than those in monotherapy (13.3% chemotherapy,  
46 20% radiotherapy). *Candida krusei* was the commonest isolated species, 48.6% (36/74).  
47 Head and neck (aOR, 15.09, 95%CI 3.05-74.59, p=0.00), gastrointestinal (aOR, 14.14,  
48 95%CI 2.25-88.63, p=0.00) malignancies and diabetes (aOR=3.18, 95% CI=1.03-9.77,  
49 p=0.04) were factors independently associated with oral carriage of *Candida* species.

## 51 Conclusion

52 Oral carriage of *Candida* species among cancer patients receiving treatment at ORCI is high,  
53 mainly due to *C.krusei* species. This is alarming since *C.krusei* has intrinsic resistance to  
54 fluconazole, a common antifungal agent used to manage fungal infections in adults.

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 is warranted since, different causative agents might have different profiles.

### Strengths and limitation of the study

- We have highlighted presence non *Candida albicans* species among cancer patients
- Unable to confirm species using biochemical and molecular tests
- Failure to perform antifungal susceptibility testing for patient management

### Keywords

*Candida* carriage, Cancer patients, Chemotherapy and radiotherapy, Gastrointestinal malignancy, Head and neck malignancy

## BACKGROUND

Oral carriage of *Candida* species is the major predisposing factor to oral candidiasis in immune-compromised patients(1). Cancer is mentioned as an immune-compromising condition that accounts for great morbidity and mortality(2). Globally it is estimated that 1 in every 3 persons suffers from cancer by 75 years(3). The use of radiation therapy, chemotherapy, and/or a combination of both is documented to compromise the patient's

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4 80 immune status further and thus predisposes these patients to opportunistic infections like oral  
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6 81 candidiasis(4–6). In addition, cancer therapy counteracts neutrophil's function and induces  
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8 82 neutrophil depletion, predisposing the person to fungal infections like oral candidiasis(7). It is  
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10 83 estimated that the rate of oral candidiasis among cancer patients ranges from 7 to 52%(8).

11  
12 84 Variation in the magnitude depends on the type of malignancy, whereby head and neck cancer  
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14 85 have a higher prevalence, followed by haematological malignancies(9–11). Historically, *C.*  
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16 86 *albicans* species have been the most common cause of oral candidiasis; however, recently,  
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18 87 non-*Candida albicans* species are increasingly implicated as causative agents of  
19  
20 88 candidiasis(12). The shift of species from *C. albicans* to non-*C. albicans* species can  
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22 89 potentially cause treatment challenges, especially in resource-limited areas where treatment is  
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24 90 usually empirical. In addition, studies have shown that *C. albicans* and non-*C. albicans*  
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26 91 though closely related, differ in their antifungal susceptibility profiles(7,13). Therefore  
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28 92 identifying a specific causative agent can help inpatient management.

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31 93 There is limited data on the predominant *Candida* species colonizing cancer patients  
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33 94 undergoing cancer treatment in our geographical area. Therefore, we conducted the present  
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35 95 study to determine the current prevalence of oral carriage of *Candida* species among cancer  
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37 96 patients receiving cancer treatment and evaluate the association between some factors and  
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39 97 oral carriage.  
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## 47 99 **MATERIALS AND METHODS**

### 48 49 100 **Study design and settings**

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51 101 A hospital-based cross-sectional study was conducted from July to August 2019 at Ocean  
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53 102 Road Cancer Institute (ORCI) in Dar es Salaam, Tanzania. ORCI is located along the Indian  
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55 103 Ocean in Ilala district, Dar es Salaam, Tanzania. It is a public national referral hospital for  
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57 104 cancer treatment in Tanzania. Currently, ORCI serves more than 50,000 patients, including  
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4 105 about 28,000 cancer patients, 10,000 cancer screening patients, and 12,000 non-cancer  
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6 106 patients. In addition, ORCI attends to over 15,000 clients in the outreach programs in the  
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8 107 Tanzania mainland regions.  
9

### 10 108 **Study population, sample size, and sampling procedure**

11  
12 109 Adult patients aged 18 years and above on anticancer therapy present at the clinic or ward on  
13  
14 110 the day of data collection were eligible for inclusion in the study. Participants were randomly  
15  
16 111 selected and those who consented were included in the study until the sample size was  
17  
18 112 reached. Sample size estimation was done using Kish Leslie formula for cross-sectional  
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20 113 study ( $N = z^2pq/\epsilon^2$ ); considering the prevalence of 15% (12), 95% CI and  $\epsilon$  at 0.05, the  
21  
22 114 estimated sample size was 196. To avoid underestimating candida oral carriage, we excluded  
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24 115 cancer patients who had taken antifungal agents in the past four weeks. Patients with signs  
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26 116 and symptoms of dry mouth were also excluded to avoid overestimation of oral candida  
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28 117 carriage. These included: mouth sores, thirstiness, cracks and cuts on lips, difficulty in  
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30 118 swallowing and loss of sense of taste.  
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### 35 119 **Data collection**

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38 120 Data collection was conducted by two research assistants who were medical doctors trained  
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40 121 on the study protocol. A well-structured questionnaire was used to collect socio-demographic  
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42 122 information such as age, sex, education status, employment status, and clinical information,  
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44 123 including the type of malignancy, type of anticancer treatment, stage of malignancy,  
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46 124 salivation status, inpatient and outpatient services and diabetes status.  
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### 50 125 **Sample collection and laboratory procedures**

51  
52 126 Oral swabs were collected from each participant as per standard procedures. Briefly, a sterile  
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54 127 cotton wool swab was used to collect the sample from the mouth of the patient. Swabs were  
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56 128 not taken from oral lesions to avoid overestimating oral carriage. After collection, the  
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3 129 samples were transported to Muhimbili University of Health and Allied Sciences (MUHAS)  
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6 130 and processed in a Microbiology laboratory.  
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8  
9 131 The oral swabs were inoculated into Sabouraud dextrose agar (SDA) media supplemented  
10  
11 132 with 50 mg/ml gentamicin and 50 mg/ml chloramphenicol (Oxoid, Basingstoke RG24 8PW,  
12  
13 133 UK) and chromogenic candida agar (CHROMagar Candida Oxoid). All media were  
14  
15 134 incubated aerobically at 37°C for 24-48 hours for phenotypic identification of *Candida*  
16  
17 135 species. *Candida* species were identified based on colour and colonial morphology on  
18  
19 136 CHROMagar as per the manufacturer's instructions. *C.albicans* isolates were further  
20  
21 137 confirmed by germ tube test. Growth of colonies in less than three quadrants of the plate and  
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23 138 absence of pseudohyphae in Gram stain indicated oral candida carriage.  
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### 31 140 **Quality control**

32  
33 141 All the reagents were prepared following the manufactures instructions. In addition, we  
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35 142 performed sterility and performance tests to check for the quality of prepared media.  
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### 39 143 **Variables**

40  
41  
42 144 The independent variables were, age, sex, education status, employment status, type of  
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44 145 malignancy, type of anticancer treatment, stage of malignancy, treatment services (inpatient  
45  
46 146 or outpatient) and diabetes status. The dependent variable was detection of candida species by  
47  
48 147 phenotypic method.  
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### 52 148 **Statistical analysis**

53  
54 149 We used STATA version 15.1 software for statistical analysis. Continuous variables were  
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56 150 summarized as the median and interquartile range (IQR), while proportions were used to  
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4 151 describe categorical variables. Group differences were determined using Fisher's exact test  
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6 152 for categorical variables. Binary logistic regression was performed to identify factors  
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8 153 associated with oral colonization. In addition, multivariable logistic regression was performed  
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10 154 to examine the associations between the outcome variable and independent variables after  
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12 155 adjustment for other variables. At a 95% confidence level, factors with a *p-value* <0.05 were  
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15 156 considered statistically significant.

### 17 157 **Patient and public involvement**

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19  
20 158 This study was designed to investigate the prevalence of oral candida carriage and the  
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22 159 causative agents among cancer patients to better plan infection prevention and control  
23  
24 160 practices, thus improving patient care. Patients were not involved in designing this research,  
25  
26 161 however, the proposed study was presented to the members of the department of  
27  
28 162 Microbiology and immunology of Muhimbili University of Health and Allied Sciences  
29  
30 163 before the recruitment of participants began. Patients who were colonized with *Candida*  
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32 164 species were notified as soon as the sample processing was complete and the final report  
33  
34 165 was communicated to the hospital management and the infection prevention and control  
35  
36 166 team of Ocean Road Cancer Institute.

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## 41 169 **RESULTS**

### 43 170 **Socio-demographic and clinical characteristics**

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45  
46 171 A total of 196 cancer patients with a mean age of 54 years, a standard deviation (SD)  $\pm$  14.2,  
47  
48 172 were enrolled in the study. Of the 196 participants, 69.9% were female, and nearly half  
49  
50 173 (87/196, 44.4%) had acquired primary education. The majority, 143/196 (73%) of the  
51  
52 174 participants, were inpatients, and about three-quarters, 151/196 (77%), received both  
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54 175 chemotherapy and radiotherapy treatment. Head and neck cancers were the most prevalent  
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56 176 type of malignancies 100/196 (51%), whereas only a few participants had gastrointestinal  
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177 cancer 7/196 (8.7%). Many participants were either in stage 2 (78/196;39.8%) or stage 3  
 178 (73/196;37.2%). Twenty-two participants ( 11.2%) had diabetes (Table 1).

179

180 **Table 1:Distribution of socio-demographic and clinical characteristics among cancer**  
 181 **patients (N = 196)**

Variable	Total number(N)	Percentage (%)
<b>Age group (Mean =54; SD± 14.2)</b>		
<54	101	51.5
>54	95	48.5
<b>Gender</b>		
Male	59	30.1
Female	137	69.9
<b>Educational level</b>		
Primary	87	44.4
Secondary and above	80	40.8
Non formal	29	14.8
<b>Smoking</b>		
No	165	84.2
Yes	31	15.8
<b>Oral hygiene practices</b>		
Frequency of tooth brushing		
1 time a day	4	2
2 times a day	192	98
Tooth cleaning material		
Mouth wash	0	0
Toothpaste	196	100
Type of tooth brush		
Plastic	196	100
Chewing stick	0	0
<b>Denture</b>		
No	196	100
Yes	0	0
<b>Patient care</b>		
Inpatient	143	73.0
Outpatient	53	27.0
<b>Treatment type</b>		
Chemotherapy	30	15.3
Radiotherapy	15	7.7
Chemotherapy and Radiotherapy	151	77.0
<b>Type of Malignancy</b>		
Head and neck*	100	51.0
Gastrointestinal	17	8.7
Breast,cervical&prostate	26	13.3
Other	53	27.0
<b>Cancer stage</b>		
1	29	14.8
2	78	39.8
3	73	37.2
4	16	8.2



<b>Diabetes status</b>		
No	174	88.8
Yes	22	11.2

182 *Others: leukemia, lymphoma, liver, kaposi sarcoma, \* also includes oropharyngeal cancer*  
 183 *and oral squamous cell carcinoma*

### 185 **Prevalence of oral colonization of *Candida* species**

186 All 196 cultured plates showed growth in less than three quadrants making the overall  
 187 prevalence of oral colonization of *Candida* species to be 37.8% (74/196). A higher carriage  
 188 rate of 44.4% (67/151) was observed in patients treated with both chemotherapy and  
 189 radiotherapy compared to each treatment separately; 13.3% (4/30) and 20% (3/15) for  
 190 chemotherapy and radiotherapy respectively ( $p=0.02$ ). Patients with head and neck  
 191 malignancies had a higher oral carriage, 54% (54/100) of *Candida* species, than other types  
 192 of malignancies ( $p<0.0001$ ). Although not statistically significant, detection of *Candida*  
 193 species was more prevalent among diabetic patients than non-diabetic; 54.5 % (12/22) vs.  
 194 35.6 % (62/174) ( $p=0.08$ ). There was no difference in the carriage rate of *Candida* species in  
 195 other parameters such as age, gender, smoking habits, education level, and cancer stage  
 196 (Table 2).

198 **Table 2: Prevalence of oral candida carriage among cancer patients by social-**  
 199 **demographic and clinical factors**

<b>Variable</b>	<b>Total number</b>	<b>Candida colonization n (%)</b>	<b>P-value</b>
<b>Overall</b>	<b>196</b>	<b>74 (37.8)</b>	
<b>Age group</b>			
<54	101	41 (40.6)	0.46
>54	95	33 (34.7)	
<b>Gender</b>			
Male	59	18 (30.5)	0.20
Female	137	56 (40.9)	
<b>Educational level</b>			
Primary	87	38 (43.7)	
Secondary and above	80	29 (36.3)	0.26

Non formal	29	7 (24.1)	
<b>Smoking</b>			
No	165	62 (35.6)	0.90
Yes	31	12 (38.7)	
<b>Patient care</b>			
Inpatient	143	52 (36.4)	0.51
Outpatient	53	22 (41.5)	
<b>Treatment type</b>			
Chemotherapy	30	4 (13.3)	
Radiotherapy	15	3 (20.0)	<b>0.02</b>
Chemotherapy and Radiotherapy	151	67 (44.4)	
<b>Type of Malignancy</b>			
Head and neck*	100	54 (54.0)	
Gastrointestinal	17	8 (47.1)	
Breast, cervical& Prostate	26	2 (7.7)	<b>&lt; 0.01</b>
Other	53	10 (18.9)	
<b>Cancer stage</b>			
1	29	10 (34.5)	
2	78	29 (37.2)	0.85
3	73	30 (41.1)	
4	16	5 (31.3)	
<b>Diabetes status</b>			
No	174	62 (35.6)	0.08
Yes	22	12 (54.5)	

200 *Others: leukemia, lymphoma, liver, kaposi sarcoma; In bold p-value of less than 0.05 that indicates*  
 201 *statistically significant association (Fisher's exact test), \* also includes oropharyngeal cancer and oral*  
 202 *squamous cell carcinoma*

### 204 **Candida species isolated from cancer patients**

205 A total of 74 patients had one type of *Candida* spp. in their oral cavity, making 74 candida  
 206 isolates. Of the 74 *Candida* spp isolated, 61(82.4%) were non-*C. albicans*. *Candida krusei*  
 207 was the dominant species accounting for 48.6% (36/74), followed by *Candida tropicalis*  
 208 (33.8%, 25/74) and lastly, *C. albicans* (17.6%, 13/74) (Figure 1).

### 210 **Predictors of Candida species oral colonization**

211 On bivariate analysis, participants receiving both chemotherapy and radiotherapy treatment  
 212 were five times more likely to have oral carriage of candida than other treatment types  
 213 (cOR5.18, 95%CI 1.72-15.58,  $p < 0.0001$ ). Likewise, in comparison to breast, cervical and

214 prostate malignancies, patients with head and neck malignancies (cOR,14.09, 95%CI 3.16-  
 215 62.83,  $p<0.0001$ ) and those with gastrointestinal cancer (cOR, 10.67, 95%CI 1.89-60.08,  
 216  $p=0.01$ ) had an increased probability of having oral carriage of *Candida spp* (Table 3).

217 After adjusting the effect of confounding factors on multivariable analysis, some types of  
 218 malignancies remained associated with the oral carriage of *Candida* species among cancer  
 219 patients. Participants with head and neck malignancies were 15 more likely (aOR, 15.09,  
 220 95%CI 3.05-74.59,  $p<0.0001$ ) to have oral carriage of *Candida* species, while those with  
 221 gastrointestinal cancer were fourteen more likely (aOR, 14.14, 95%CI 2.25-88.63,  $p<0.0001$ )  
 222 to have candidiasis compared to those with breast, cervical and prostate malignancies. In  
 223 addition, the probability of being colonized by *Candida* species was three times higher  
 224 among diabetic patients than non-diabetic patients (aOR=3.18, 95% CI=1.03-9.77,  $p=0.04$ )  
 225 (Table 3).

227 **Table 3: Bivariate and Multivariate logistic regression for the factors associated with**  
 228 **candida oral carriage**

Variable	Detection of <i>Candida</i> spp, n (%)	Univariate cOR	95% CI	p-value	Multivariate aOR	95% CI	p-value
<b>Gender (p=0.2)</b>							
Male	18 (30.5)	Ref			Ref		
Female	56 (40.9)	1.57	0.82-3.02	0.21	1.69	0.82-3.49	0.16
<b>Treatment type (p=0.02)</b>							
Chemotherapy	4 (13.3)	Ref			Ref		
Radiotherapy	3 (20.0)	1.63	0.31-8.43	0.56	1.97	0.31-12.55	0.47
Chemotherapy and Radiotherapy	67 (44.4)	5.18	1.72-15.58	<0.01	2.22	0.52-9.56	0.28
<b>Type of Malignancy (p=0.00)</b>							
Head and neck*	54 (54.0)	14.09	3.16-62.83	<0.01	15.09	3.05-74.59	<0.01
Gastrointestinal	8 (47.1)	10.67	1.89-60.08	0.01	14.14	2.25-88.63	<0.01
Breast, cervical & Prostate	2 (7.7)	Ref			Ref		
Other	10 (18.9)	2.79	0.56-13.80	0.21	4.45	0.80-24.98	0.09
<b>Diabetes status</b>							

<b>(p=0.08)</b>							
No	62 (35.6)	Ref			Ref		
Yes	12 (54.8)	2.17	0.89-5.30	0.08	3.18	1.03-9.77	<b>0.04</b>

229 *cOR* stands for crude odd ratio(Binary logistic regression), *aOR* stands for adjusted odd ratio (Log  
 230 likelihood)and *Ref* stands for referenceassociation,\* also includes oropharyngeal cancer and oral  
 231 squamous cell carcinoma

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## 234 DISCUSSION

235 Oral candidiasis, which is usually preceded by colonization, is a problem among  
 236 immunocompromised patients with cancer, especially in cytotoxic therapy. In the present  
 237 study, we report a prevalence of oral *Candida* species colonization among cancer patients at  
 238 ORCI undergoing chemotherapy and/or radiotherapy to be 37.8%. Our finding is slightly  
 239 higher compared to 25%, which was reported in Nagasaki, Japan, by Kawashita, Y *et al.*,  
 240 2011(14), and 30.1% reported in France by Grigorov J *et al.*,2010(15). The high prevalence  
 241 reported here might be attributed by the high number of patients who had advanced stage (2  
 242 and 3) of cancer. In the present study majority of participants were either in stage 2 (37.2%)  
 243 or stage 3 (41.1%) of cancer making them more prone to oral candida carriage. On the other  
 244 hand, Al-Abeid *et al.*, 2004 reported a much higher prevalence of candida colonization, i.e.,  
 245 72.6% in Jordanian cancer patients(16). The observed differences may be attributed to  
 246 geographical location, population characteristics, and sampling protocol. There is limited  
 247 literature on oral candida carriage in the study settings, both locally and nearby geographical  
 248 area. However, the prevalence of oral candida carriage has been reported to be 10.3%  
 249 among people living with HIV in Mwanza, 10.3% while that of the control group in the same  
 250 study was reported to be 4.5% (12). Different methodological approaches might be a  
 251 contributing factor for the observed difference, whereby in a study conducted in Mwanza  
 252 used more sensitive test (Matrix-assisted laser desorption ionization-time of flight mass

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4 253 spectrometry) for confirmation of candida isolates versus the use of CHROMagar in the  
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6 254 present study which might have overestimated the reported prevalence. Furthermore, our  
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8 255 study participants were on either chemotherapy and/or radiotherapy which is a risk factor  
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10 256 for oral candida colonization compared to the population used in Mwanza who were not in  
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13 257 such therapy.

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15 258 The role of cell-mediated host immunity (CMI) in controlling fungal infections is well  
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17 259 known. Scientific evidence shows that cytotoxic chemotherapy and radiation used in treating  
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19 260 malignancies compromises CMI, thus predisposing a person to fungal infections. In the  
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22 261 current study, nearly all patients who had head and neck malignancy received radiotherapy  
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24 262 and chemotherapy. Oral colonization was highest in this group (54.0%) among all patients.  
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26 263 This result is comparable to the studies done by Lone M Set *al.*, who found oral candidiasis  
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28 264 was highest in head & neck cancer patients compared to other types of malignancies(11).

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31 265 In the present study higher colonization rate (44.4%) was seen in patients receiving  
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33 266 chemotherapy and radiotherapy together than in patients receiving monotherapy (either  
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35 267 chemotherapy or radiation therapy). Similar results were obtained in a study conducted by  
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37 268 Manish Jain *et al.*, 2016 which observed a significant increase in oral carriage of *Candida*  
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39 269 species in patients taking both radiation and chemotherapy(1). This observation may be  
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42 270 explained by the fact that cytotoxic drugs given during chemotherapy cause dryness of oral  
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45 271 mucosa facilitating infections by various pathogens, including fungi, and at the same time,  
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47 272 radiation causes mucositis and changes in salivary glands, which lead to quantitative and  
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50 273 qualitative changes in saliva, whereby thick saliva makes the oral environment conducive for  
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52 274 fungal colonization(17). Hence, taken together, these factors increase the chances of fungal  
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54 275 colonization.

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56 276 Other researchers have identified *Candida albicans* as the most common species causing oral  
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58 277 colonization(10,18). However, this was not the case in this study; we report *Candida krusei*

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4 278 as the predominant species detected in our study setting. In addition, the predominance of  
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6 279 *Candida krusei* colonizing patients on cancer therapy in the area where fluconazole is the  
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8 280 main therapy is alarming. This is because *Candida krusei* has intrinsic resistance to  
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10 281 fluconazole(12). We also report the detection of *Candida tropicalis* which has been  
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12 282 associated with a high predictive value for invasive fungal infection(19).

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15 283 These results are worrisome as colonization is a risk factor for infection, putting colonized  
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17 284 patients at risk of subsequent infection. Therefore, detection of non- *C. albicans* species,  
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19 285 especially *Candida krusei*, emphasizes the need for species identification and drug  
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21 286 susceptibility testing of the infecting *Candida* species in cancer patients before starting  
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23 287 empirical therapy.

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26 288 This study had some limitations; we did not collect information about some variables which  
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28 289 could affect oral candida carriage. These variables include; duration of cancer treatment,  
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30 290 prolonged use of antibiotics, history of dental caries and periodontal diseases. Furthermore  
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32 291 we did not perform biochemical tests and molecular tests to further confirm/differentiate  
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34 292 *Candida* species. Also antifungal susceptibility testing was not performed in the present  
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36 293 study to show the antifungal profile among different *Candida* species. Nonetheless, the study  
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38 294 has shown the contribution of non-*C.albicans* in the oral cavity of cancer patients which  
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40 295 could potentially lead to subsequent infections which might be difficult to treat due to their  
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42 296 intrinsic resistance to conventional antifungal agents.

## 43 44 45 46 47 297 **CONCLUSION**

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49 298 Oral non-*Candida* species colonization is high among cancer patients at ORCI. Patients with  
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51 299 head and neck malignancies are at high risk of colonization, a risk factor for subsequent  
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53 300 infections. There is therefore, a need for prompt identification of causative agents of  
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55 301 candidiasis among cancer patients and fungal susceptibility testing for better management of  
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57 302 patients as resistance pattern differs between *C. albicans* and non-*C. albicans* species.  
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## **LIST OF ABBREVIATIONS**

**AIDS-** Acquired Immunodeficiency Syndrome, **HIV-** Human Immunodeficiency Virus,

**OC-** Oral Candidiasis, **ORCI-** Ocean Road Cancer Institute

## **DECLARATIONS**

### **Ethics approval and consent to participate**

Ethical clearance was obtained from the Senate of Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS), Ref.No. DA.25/111/01C.

Permission to conduct the study was obtained from the ORCI administration. Before enrolling in the study, written informed consent was obtained from each participant.

Confidentiality of the study participants was ensured using codes instead of participants'names.

### **Consent for Publication**

Not applicable

### **Availability of data and materials**

All relevant data generated and analyzed during this study are included in this manuscript.

### **Competing Interests**

The authors declare that they have no competing interests.

### **Funding**

No funding was received for this study.

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4 329 **Authors' contributions**

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6 330 UK and DK were involved in the study's conceptualization and performed data collection and  
7  
8 331 laboratory work. UK, DK, and AMM performed all the statistical analyses. UK, DK, AMM,  
9  
10 332 MFM, and MM were involved in drafting the manuscript. JM and MM were involved in a  
11  
12 333 critical review of the manuscript.  
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15 334

16 335 **Acknowledgment**

17  
18 336 The authors would like to acknowledge all patients who participated in this study.  
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22 338 **REFERENCES**

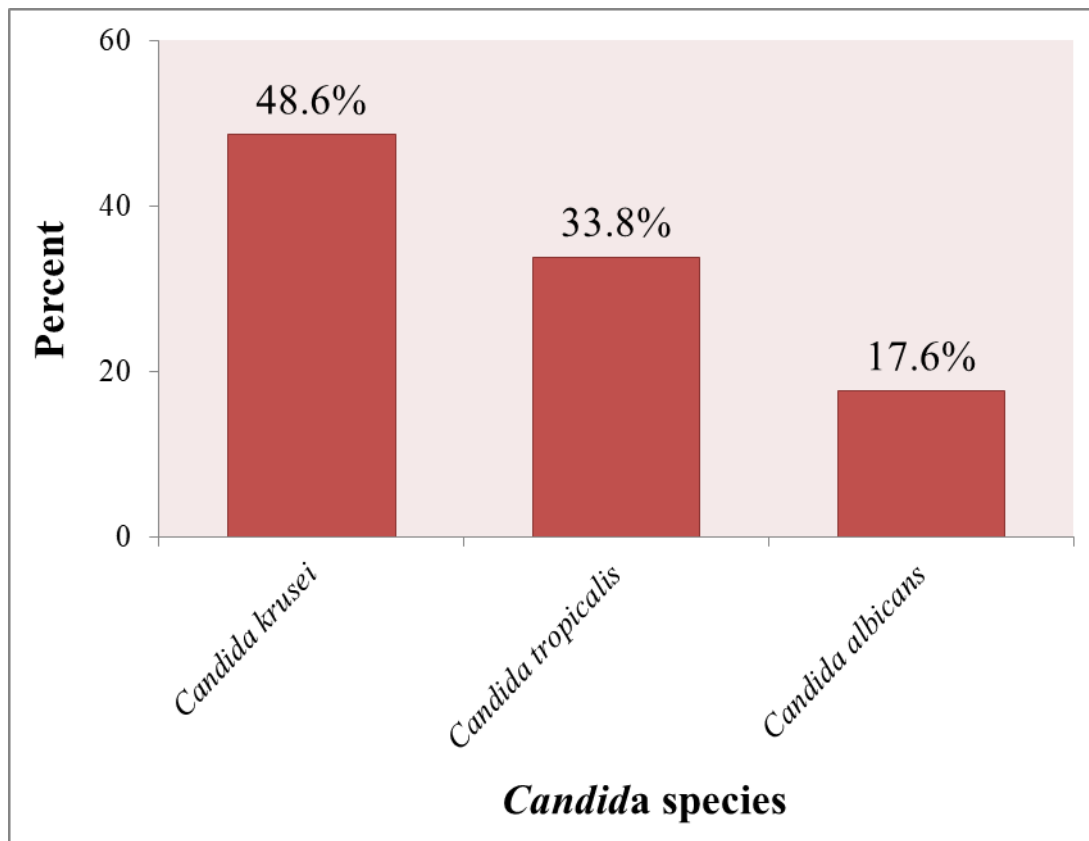
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Figure 1: Distribution of *Candida* species isolates from cancer patients



The figure illustrates the distribution of specific *Candida* species isolates that were obtained from 196 cancer patients at Ocean road Cancer Institute (ORCI).

**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cross-sectional studies**

Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Page 1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Pages 2 and 3
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	Page 5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 6
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 6
Bias	9	Describe any efforts to address potential sources of bias	Page 5
Study size	10	Explain how the study size was arrived at	Page 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pages 6 and 7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Pages 6 and 7
		(b) Describe any methods used to examine subgroups and interactions	NA
		(c) Explain how missing data were addressed	NA
		(d) If applicable, describe analytical methods taking account of sampling strategy	NA
		(e) Describe any sensitivity analyses	NA
<b>Results</b>			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pages 7 and 8
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	Pages 8 and 9
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 10
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	Page 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pages 11 and 12
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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# BMJ Open

## The predominance of non-*Candida albicans* species oral colonisation among patients on anticancer therapy: Findings from a cross-sectional study in Tanzania

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-070003.R2
Article Type:	Original research
Date Submitted by the Author:	31-Mar-2023
Complete List of Authors:	Kibwana, Upendo; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Manyahi, Joel; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Kamori, Doreen; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Mushi, Martha; Catholic University of Health and Allied Sciences, Department of Microbiology and Immunology Mwandigha, Ambele M.; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology Majigo, Mtebe; Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology
<b>Primary Subject Heading</b>:	Diagnostics
Secondary Subject Heading:	Infectious diseases
Keywords:	MICROBIOLOGY, INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES, MYCOLOGY, Head & neck tumours < ONCOLOGY

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4 1 **The predominance of non-*Candida albicans* species oral colonisation among patients on**  
5 2 **anticancer therapy: Findings from a cross-sectional study in Tanzania**

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## 28 ABSTRACT

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

32 **Design:** A hospital-based cross-sectional study

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

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

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

42 **Results:** A total of 196 participants were enrolled in the study. The overall oral carriage of  
43 *Candida* species was 37.8% (74/196). The prevalence was higher among patients undergoing  
44 chemotherapy and radiotherapy (44.4%) than those in monotherapy (13.3% chemotherapy,  
45 20% radiotherapy). *Candida krusei* was the commonest isolated species, 48.6% (36/74).  
46 Head and neck (aOR, 15.09, 95%CI 3.05-74.59, p=0.00), gastrointestinal (aOR, 14.14,  
47 95%CI 2.25-88.63, p=0.00) malignancies and diabetes (aOR=3.18, 95% CI=1.03-9.77,  
48 p=0.04) were factors independently associated with oral carriage of *Candida* species.

## 50 Conclusion

51 The oral carriage of *Candida* species among cancer patients receiving treatment at ORCI is  
52 high, mainly due to *C.krusei* species. This is alarming since *C.krusei* has intrinsic resistance

1  
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3  
4 53 to fluconazole, a common antifungal agent used to manage adult fungal infections. Therefore,  
5  
6 54 efforts should be put into conducting regular check-ups for such opportunistic pathogens as  
7  
8 55 they can lead to subsequent infections. Furthermore, studies conducted to determine the  
9  
10 56 antifungal profile of the causative agents are warranted since different causative agents might  
11  
12  
13 57 have different profiles.

#### 14 15 58 **Strengths and limitations of the study**

- 16  
17 59 • We used chromogenic media for candida speciation, which can be adopted in other  
18  
19 60 resource-limited settings to provide preliminary identification of Candida species for  
20  
21 61 proper patient management
- 22  
23 62 • Unable to confirm species using biochemical and molecular tests
- 24  
25 63 • Failure to perform antifungal susceptibility testing for patient management

#### 26 27 28 65 **Keywords**

29  
30 66 Candida carriage, Cancer patients, Chemotherapy and radiotherapy, Gastrointestinal  
31  
32  
33 67 malignancy, Head and neck malignancy

## 79 BACKGROUND

80 Oral carriage of *Candida* species is the major predisposing factor to oral candidiasis in  
81 immune-compromised patients(1). Cancer is mentioned as an immune-compromising  
82 condition that accounts for significant morbidity and mortality(2). Globally it is estimated  
83 that 1 in every 3 persons will get cancer by the age of 75 years(3). The use of radiation  
84 therapy, chemotherapy, and a combination of both is documented to compromise the patient's  
85 immune status further and thus predisposes these patients to opportunistic infections like oral  
86 candidiasis(4–6). In addition, cancer therapy counteracts neutrophil function and induces  
87 neutrophil depletion, predisposing the person to fungal infections like oral candidiasis(7). The  
88 rate of oral candidiasis among cancer patients is estimated to be 7 to 52%(8).

89 Variation in the magnitude depends on the type of malignancy, whereby head and neck  
90 cancer have a higher prevalence, followed by haematological malignancies(9–11).  
91 Historically, *C. albicans* species have been the most common cause of oral candidiasis;  
92 however, recently, non-*Candida albicans* species are increasingly implicated as causative  
93 agents of candidiasis(12). The shift of species from *C. albicans* to non-*C. albicans* species  
94 can potentially cause treatment challenges, especially in resource-limited areas where  
95 treatment is usually empirical. In addition, studies have shown that *C. albicans* and non-*C.*  
96 *albicans* though closely related, differ in their antifungal susceptibility profiles(7,13).  
97 Therefore, identifying a specific causative agent can help inpatient management.

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

## 103 MATERIALS AND METHODS

### 104 Study design and settings

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

### 112 Study population, sample size, and sampling procedure

113 Adult patients aged 18 years and above on anticancer therapy present at the clinic or ward on  
114 the day of data collection were eligible for inclusion in the study. Participants were randomly  
115 selected, and those consented were included in the study until the sample size was reached.

116 Sample size estimation was done using the Kish Leslie formula for cross-sectional study ( $N = z^2pq/\epsilon^2$ ); considering the prevalence of 15% (12), 95% CI and  $\epsilon$  at 0.05, the estimated  
117 sample size was 196. To avoid underestimating candida oral carriage, we excluded cancer  
118 patients who had taken antifungal agents in the past four weeks. In addition, patients with  
119 signs and symptoms of dry mouth were also excluded to avoid overestimating oral candida  
120 carriage. These included: mouth sores, thirstiness, cracks and cuts on lips, difficulty  
121 swallowing and loss of sense of taste.

### 123 Data collection

124 Data were collected by two research assistants who were medical doctors trained in the study  
125 protocol. A well-structured questionnaire was used to collect socio-demographic information  
126 such as age, sex, education status, employment status, and clinical data, including the type of

1  
2  
3  
4 127 malignancy, type of anticancer treatment, stage of malignancy, salivation status, inpatient and  
5  
6 128 outpatient services and diabetes status.

### 8 129 **Sample collection and laboratory procedures**

10  
11 130 Oral swabs were collected from each participant as per standard procedures. Briefly, a sterile  
12  
13 131 cotton wool swab was used to collect the sample from the mouth of the patient. Swabs were  
14  
15 132 not taken from oral lesions to avoid overestimating oral carriage. After collection, the  
16  
17 133 samples were transported to Muhimbili University of Health and Allied Sciences (MUHAS)  
18  
19 134 and processed in a Microbiology laboratory.

22  
23 135 The oral swabs were inoculated into Sabouraud dextrose agar (SDA) media supplemented  
24  
25 136 with 50 mg/ml gentamicin and 50 mg/ml chloramphenicol (Oxoid, Basingstoke RG24 8PW,  
26  
27 137 UK) and chromogenic candida agar (CHROMagar Candida Oxoid). All media were  
28  
29 138 incubated aerobically at 37°C for 24-48 hours for phenotypic identification of *Candida*  
30  
31 139 species. *Candida* species were identified based on colour and colonial morphology on  
32  
33 140 CHROMagar as per the manufacturer's instructions. *C.albicans* isolates were further  
34  
35 141 confirmed by germ tube test. Growth of colonies in less than three quadrants of the plate and  
36  
37 142 absence of pseudo hyphae in Gram stain indicated oral candida carriage.

### 42 43 143 **Quality control**

44  
45 144 All the reagents were prepared following the manufactures instructions. In addition, we  
46  
47 145 performed sterility and performance tests to check for the quality of the prepared media.

### 49 50 146 **Variables**

51  
52  
53 147 The independent variables were age, sex, education status, employment status, type of  
54  
55 148 malignancy, type of anticancer treatment, stage of malignancy, treatment services (inpatient

1  
2  
3  
4 149 or outpatient) and diabetes status. The dependent variable was the detection of candida  
5  
6 150 species by the phenotypic method.  
7  
8

### 9 151 **Statistical analysis**

10  
11 152 We used STATA version 15.1 software for statistical analysis. Continuous variables were  
12  
13 153 summarised as the median and interquartile range (IQR), while proportions were used to  
14  
15 154 describe categorical variables. Group differences were determined using Fisher's exact test  
16  
17 155 for categorical variables. Binary logistic regression was performed to identify factors  
18  
19 156 associated with oral colonisation. In addition, multivariable logistic regression was performed  
20  
21 157 to examine the associations between the outcome variable and independent variables after  
22  
23 158 adjustment for other variables. At a 95% confidence level, factors with a *p-value* <0.05 were  
24  
25 159 considered statistically significant.  
26  
27  
28  
29

### 30 160 **Patient and public involvement**

31 161 Patients or the public were not involved in the design, or conduct, or reporting, or  
32  
33 162 dissemination plans of our research study.  
34  
35  
36  
37

38 163

## 39 164 **RESULTS**

### 40 165 **Socio-demographic and clinical characteristics**

41  
42  
43 166 A total of 196 cancer patients with a mean age of 54 years, a standard deviation (SD)  $\pm$  14.2,  
44  
45 167 were enrolled in the study. Of the 196 participants, 69.9% were female, and nearly half  
46  
47 168 (87/196, 44.4%) had acquired primary education. The majority, 143/196 (73%) of the  
48  
49 169 participants, were inpatients, and about three-quarters, 151/196 (77%), received both  
50  
51 170 chemotherapy and radiotherapy treatment. Head and neck cancers were the most prevalent  
52  
53 171 type of malignancies 100/196 (51%), whereas only a few participants had gastrointestinal  
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172 cancer 7/196 (8.7%). Many participants were either in stage 2 (78/196;39.8%) or stage 3  
 173 (73/196;37.2%). Twenty-two participants ( 11.2%) had diabetes (Table 1).

174

175 **Table 1: Distribution of sociodemographic and clinical characteristics among cancer**  
 176 **patients (N = 196)**

Variable	Total number(N)	Percentage (%)
<b>Age group (Mean =54; SD± 14.2)</b>		
<54	101	51.5
>54	95	48.5
<b>Gender</b>		
Male	59	30.1
Female	137	69.9
<b>Educational level</b>		
Primary	87	44.4
Secondary and above	80	40.8
Non formal	29	14.8
<b>Smoking</b>		
No	165	84.2
Yes	31	15.8
<b>Oral hygiene practices</b>		
Frequency of tooth brushing		
1 time a day	4	2
2 times a day	192	98
Tooth cleaning material		
Mouth wash	0	0
Toothpaste	196	100
Type of toothbrush		
Plastic	196	100
Chewing stick	0	0
<b>Denture</b>		
No	196	100
Yes	0	0
<b>Patient care</b>		
Inpatient	143	73.0
Outpatient	53	27.0
<b>Treatment type</b>		
Chemotherapy	30	15.3
Radiotherapy	15	7.7
Chemotherapy and Radiotherapy	151	77.0
<b>Type of Malignancy</b>		
Head and neck*	100	51.0
Gastrointestinal	17	8.7
Breast, cervical & prostate	26	13.3
Other	53	27.0
<b>Cancer stage</b>		
1	29	14.8
2	78	39.8
3	73	37.2
4	16	8.2
<b>Diabetes status</b>		

No	174	88.8
Yes	22	11.2

177 *Others: leukaemia, lymphoma, liver, kaposi sarcoma, \* also includes oropharyngeal cancer*  
 178 *and oral squamous cell carcinoma*  
 179

## 180 Prevalence of oral colonisation of *Candida* species

181 All 196 cultured plates showed growth in less than three quadrants making the overall  
 182 prevalence of oral colonisation of *Candida* species to be 37.8% (74/196). A higher carriage  
 183 rate of 44.4% (67/151) was observed in patients treated with both chemotherapy and  
 184 radiotherapy compared to each treatment separately; 13.3% (4/30) and 20% (3/15) for  
 185 chemotherapy and radiotherapy, respectively ( $p=0.02$ ). Patients with head and neck  
 186 malignancies had a higher oral carriage, 54% (54/100) of *Candida* species, than other types  
 187 of malignancies ( $p<0.0001$ ). Although not statistically significant, the detection of *Candida*  
 188 species was more prevalent among diabetic patients than non-diabetic; 54.5 % (12/22) vs  
 189 35.6 % (62/174) ( $p=0.08$ ). There was no difference in the carriage rate of *Candida* species in  
 190 other parameters such as age, gender, smoking habits, education level, and cancer stage  
 191 (Table 2).

193 **Table 2: Prevalence of oral candida carriage among cancer patients by social-**  
 194 **demographic and clinical factors**

Variable	Total number	Candida colonisation n (%)	P-value
<b>Overall</b>	<b>196</b>	<b>74 (37.8)</b>	
<b>Age group</b>			
<54	101	41 (40.6)	0.46
>54	95	33 (34.7)	
<b>Gender</b>			
Male	59	18 (30.5)	0.20
Female	137	56 (40.9)	
<b>Educational level</b>			
Primary	87	38 (43.7)	
Secondary and above	80	29 (36.3)	0.26
Non formal	29	7 (24.1)	
<b>Smoking</b>			
No	165	62 (35.6)	0.90
Yes	31	12 (38.7)	



<b>Patient care</b>			
Inpatient	143	52 (36.4)	0.51
Outpatient	53	22 (41.5)	
<b>Treatment type</b>			
Chemotherapy	30	4 (13.3)	
Radiotherapy	15	3 (20.0)	<b>0.02</b>
Chemotherapy and Radiotherapy	151	67 (44.4)	
<b>Type of Malignancy</b>			
Head and neck*	100	54 (54.0)	
Gastrointestinal	17	8 (47.1)	
Breast, cervical& Prostate	26	2 (7.7)	<b>&lt; 0.01</b>
Other	53	10 (18.9)	
<b>Cancer stage</b>			
1	29	10 (34.5)	
2	78	29 (37.2)	0.85
3	73	30 (41.1)	
4	16	5 (31.3)	
<b>Diabetes status</b>			
No	174	62 (35.6)	0.08
Yes	22	12 (54.5)	

195 *Others: leukaemia, lymphoma, liver, kaposi sarcoma; In bold p-value of less than 0.05 that*  
 196 *indicates statistically significant association (Fisher's exact test), \* also includes oropharyngeal cancer*  
 197 *and oral squamous cell carcinoma.*

198

### 199 **Candida species isolated from cancer patients**

200 A total of 74 patients had one type of *Candida* spp. in their oral cavity, making 74 candida  
 201 isolates. Of the 74 *Candida* spp isolated, 61(82.4%) were non-*C. albicans*. *Candida krusei*  
 202 was the dominant species accounting for 48.6% (36/74), followed by *Candida tropicalis*  
 203 (33.8%, 25/74) and lastly, *C. albicans* (17.6%, 13/74) (Figure 1).

### 204 **Predictors of Candida species oral colonisation**

205 On bivariate analysis, participants receiving both chemotherapy and radiotherapy treatment  
 206 were five times more likely to have oral carriage of candida than other treatment types  
 207 (cOR5.18, 95%CI 1.72-15.58,  $p<0.0001$ ). Likewise, in comparison to breast, cervical and  
 208 prostate malignancies, patients with head and neck malignancies (cOR,14.09, 95%CI 3.16-  
 209 62.83,  $p<0.0001$ ) and those with gastrointestinal cancer (cOR, 10.67, 95%CI 1.89-60.08,  
 210  $p=0.01$ ) had an increased probability of having oral carriage of *Candida* spp (Table 3).

211 After adjusting the effect of confounding factors on multivariable analysis, some types of  
 212 malignancies remained associated with the oral carriage of *Candida* species among cancer  
 213 patients. Participants with head and neck malignancies were 15 more likely (aOR, 15.09,  
 214 95%CI 3.05-74.59,  $p<0.0001$ ) to have oral carriage of *Candida* species, while those with  
 215 gastrointestinal cancer were fourteen more likely (aOR, 14.14, 95%CI 2.25-88.63,  $p<0.0001$ )  
 216 to have candidiasis as compared to those with breast, cervical and prostate malignancies. In  
 217 addition, the probability of being colonised by *Candida* species was three times higher among  
 218 diabetic patients than non-diabetic patients (aOR=3.18, 95% CI=1.03-9.77,  $p=0.04$ ) (Table  
 219 3).

221 **Table 3: Bivariate and Multivariate logistic regression for the factors associated with**  
 222 **candida oral carriage**

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

223 *cOR* stands for crude odd ratio(Binary logistic regression), *aOR* stands for adjusted odd ratio (Log-  
 224 likelihood), and *Ref* stands for reference association, \* also includes oropharyngeal cancer and oral  
 225 squamous cell carcinoma

## 227 DISCUSSION

228 Oral candidiasis, usually preceded by colonisation, is a problem among immunocompromised  
229 patients with cancer, especially in cytotoxic therapy. In the present study, we report a  
230 prevalence of oral *Candida* species colonisation among cancer patients at ORCI undergoing  
231 chemotherapy and/or radiotherapy to be 37.8%. Our finding is slightly higher compared to  
232 25%, reported in Nagasaki, Japan, by Kawashita, Y *et al.*, 2011(14), and 30.1% reported in  
233 France by Grigorov J *et al.*,2010(15). The high prevalence reported here might be attributed  
234 to the high number of patients with advanced cancer stages (2 and 3). In the present study,  
235 most participants were either in stage 2 (37.2%) or stage 3 (41.1%) of cancer, making them  
236 more prone to oral candida carriage. On the other hand, Al-Abeid *et al.*, 2004 reported a  
237 much higher prevalence of candida colonisation, i.e., 72.6% in Jordanian cancer patients(16).  
238 The observed differences may be attributed to geographical location, population  
239 characteristics, and sampling protocol.

240 The limited literature on oral candida carriage exists in the study settings; locally and in  
241 nearby geographical areas. However, the prevalence of oral candida carriage has been  
242 reported to be 10.3% among people living with HIV in Mwanza, while, that of the control  
243 group in the same study was reported to be 4.5%(12). Different methodological approaches  
244 might be a contributing factor for the observed difference, whereby a study conducted in  
245 Mwanza used a more sensitive test (Matrix-assisted laser desorption ionization-time of flight  
246 mass spectrometry) for confirmation of candida isolates versus the use of CHROMagar in the  
247 present study which might have overestimated the reported prevalence. Furthermore, our  
248 study participants were on either chemotherapy and/or radiotherapy, a risk factor for oral  
249 candida colonisation, compared to the population used in Mwanza who were not in such  
250 therapy.

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4 251 The role of cell-mediated host immunity (CMI) in controlling fungal infections is well  
5  
6 252 known. Scientific evidence shows that cytotoxic chemotherapy and radiation used in treating  
7  
8 253 malignancies compromise CMI, thus predisposing a person to fungal infections. In the  
9  
10 254 current study, nearly all patients who had head and neck malignancy received radiotherapy  
11  
12 255 and chemotherapy. As a result, oral colonisation was highest in this group (54.0%) among all  
13  
14 256 patients. This result is comparable to the studies done by Lone M Set *al.*, who found oral  
15  
16 257 candidiasis was highest in head & neck cancer patients compared to other types of  
17  
18 258 malignancies(11).

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20  
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22  
23 259 In the present study, a higher colonisation rate (44.4%) was seen in patients receiving  
24  
25 260 chemotherapy and radiotherapy together than in patients receiving monotherapy (either  
26  
27 261 chemotherapy or radiation therapy). Similar results were obtained in a study conducted by  
28  
29 262 Manish Jain *et al.*,2016 which observed a significant increase in oral carriage of *Candida*  
30  
31 263 species in patients taking both radiation and chemotherapy(1). This observation may be  
32  
33 264 explained by the fact that cytotoxic drugs given during chemotherapy cause dryness of oral  
34  
35 265 mucosa facilitating infections by various pathogens, including fungi, and at the same time,  
36  
37 266 radiation causes mucositis and changes in salivary glands, which leads to quantitative and  
38  
39 267 qualitative changes in saliva, whereby thick saliva makes the oral environment conducive for  
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41 268 fungal colonization(17). Hence, taken together, these factors increase the chances of fungal  
42  
43 269 colonisation.

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49 270 Other researchers have identified *Candida albicans* as the most common species causing oral  
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51 271 colonization(10,18). However, this was not the case in this study; we report *Candida krusei*  
52  
53 272 as the predominant species detected in our study setting. In addition, the predominance of  
54  
55 273 *Candida krusei* colonising patients on cancer therapy in the area where fluconazole is the  
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57 274 main therapy is alarming considering the intrinsic resistance of *Candida krusei* to

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4 275 fluconazole(12). We also report the detection of *Candida tropicalis*, which has been  
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6 276 associated with a high predictive value for invasive fungal infection(19).  
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9 277 These results are worrisome as colonisation is a risk factor for infection, putting colonised  
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11 278 patients at risk of subsequent infection. Therefore, the detection of non-*C. albicans* species,  
12  
13 279 especially *Candida krusei*, emphasise the need for species identification and drug  
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15 280 susceptibility testing of the infecting *Candida* species in cancer patients before starting  
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17 281 empirical therapy.  
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21 282 This study had limitations; we did not collect information about variables that could affect  
22  
23 283 oral candida carriage. These variables include; duration of cancer treatment, prolonged use of  
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25 284 antibiotics, history of dental caries and periodontal diseases. Furthermore, we did not perform  
26  
27 285 biochemical and molecular tests to confirm further/differentiate *Candida* species. Also,  
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29 286 antifungal susceptibility testing was not performed in the present study to show the antifungal  
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31 287 profile among *Candida* species. Nonetheless, the study has demonstrated the contribution of  
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33 288 non-*C. albicans* in the oral cavity of cancer patients, potentially leading to subsequent  
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35 289 infections that might be difficult to treat due to their intrinsic resistance to conventional  
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37 290 antifungal agents.  
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## 43 291 **CONCLUSION**

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45 292 Oral non-*Candida* species colonisation is high among cancer patients at ORCI. Patients with  
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47 293 head and neck malignancies are at increased risk of colonisation, a risk factor for subsequent  
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49 294 infections. There is, therefore, a need for prompt identification of causative agents of  
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51 295 candidiasis among cancer patients and fungal susceptibility testing for better management of  
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53 296 patients as resistance pattern differs between *C. albicans* and non-*C. albicans* species.  
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4 298 **LIST OF ABBREVIATIONS**

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6 299 **AIDS-** Acquired Immunodeficiency Syndrome, **HIV-** Human Immunodeficiency Virus, **OC-**  
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8 300 Oral Candidiasis, **ORCI-** Ocean Road Cancer Institute

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11 301 **DECLARATIONS**

12  
13 302 **Ethics approval and consent to participate**

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15 303 Ethical clearance was obtained from the Senate of Research and Publications Committee of  
16  
17 304 the Muhimbili University of Health and Allied Sciences (MUHAS), Ref.No. DA.25/111/01C.  
18  
19 305 Permission to conduct the study was obtained from the ORCI administration. Before  
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21 306 enrolling in the study, written informed consent was obtained from each participant. The  
22  
23 307 confidentiality of the study participants was ensured using codes instead of participants'  
24  
25 308 names.

26  
27 309 **Consent for Publication**

28  
29 310 Not applicable

30  
31 311 **Availability of data and materials**

32  
33 312 All data relevant to the study are included in the article.

34  
35 313 **Competing Interests**

36  
37 314 The authors declare that they have no competing interests.

38  
39 315 **Funding**

40  
41 316 No funding was received for this study.

42  
43 317 **Authors' contributions**

44  
45 318 UK and DK were involved in the study's conceptualisation and performed data collection and  
46  
47 319 laboratory work. UK, DK, and AMM performed all the statistical analyses. UK, DK, AMM,

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4 320 MFM, and MM were involved in drafting the manuscript. JM and MM were involved in a  
5  
6 321 critical review of the manuscript.  
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8

9 322 **Acknowledgement**

10 323 The authors would like to acknowledge all patients who participated in this study and an  
11  
12  
13 324 online writing assistant; Grammarly Inc. version 1.22.01, premium subscription for revising  
14  
15 325 the English language in this manuscript.  
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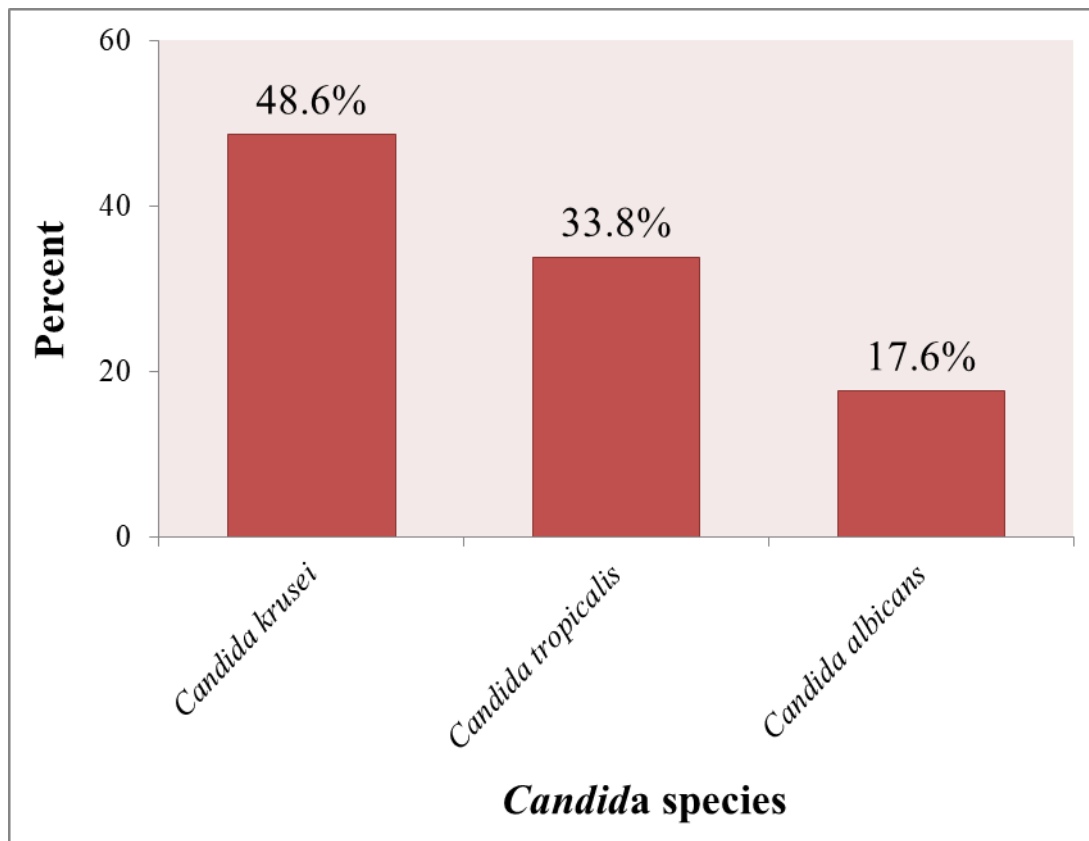
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Figure 1: Distribution of *Candida* species isolates from cancer patients



The figure illustrates the distribution of specific *Candida* species isolates that were obtained from 196 cancer patients at Ocean road Cancer Institute (ORCI).

**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cross-sectional studies**

Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Page 1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Pages 2 and 3
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	Page 5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 6
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 6
Bias	9	Describe any efforts to address potential sources of bias	Page 5
Study size	10	Explain how the study size was arrived at	Page 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pages 6 and 7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Pages 6 and 7
		(b) Describe any methods used to examine subgroups and interactions	NA
		(c) Explain how missing data were addressed	NA
		(d) If applicable, describe analytical methods taking account of sampling strategy	NA
		(e) Describe any sensitivity analyses	NA
<b>Results</b>			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pages 7 and 8
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	Pages 8 and 9
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 10
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	Page 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pages 11 and 12
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).