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High Oral Carriage of Non-*albicans Candida* spp. among HIV-infected individuals

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ABSTRACT

Background: Non-*albicans Candida* (NAC) spp. in immunocompromised patients are linked to invasive infections with narrow treatment choice. This study aimed at comparing the oral colonization of NAC spp. between HIV and non-HIV infected individuals in Mwanza, Tanzania.
Method: Oral rinse of 351 HIV-infected and 639 non-HIV infected individuals were collected between March and July 2015. Phenotypic identifications of *Candida* spp. was done using Candida Chromogenic agar and confirmed by MALDI-TOF MS.
Results: NAC spp. were detected in 36/351 (10.3%) HIV-infected individuals compared to 28/639 (4.4%) of non-HIV infected individuals; P=0.0003. In HIV infected individuals, commonly isolated NAC spp. were *Candida tropicalis*, 10(2.8%), *C. krusei* (*Issatschenki orientalis*) 9(2.6%) and *C. glabrata* 8(2.3%). While for non-HIV infected individuals *C. dubliniensis 8*(1.3%) and *C. tropicalis* 5(0.9%) were commonly detected. As CD4 count/μl decreases by one unit the risk of being colonized by NAC spp. among HIV infected individuals increases by 1% (OR 1.01, 95% CI; 1.001-1.004, P=0.001).
Conclusion: The prevalence of NAC spp. is high among HIV-infected individuals with low CD4 count placing them at higher risk of invasive infections. Further studies to investigate the role of NAC spp. in causing invasive infections among immunocompromised patients are recommended.

1. Background

Oral candidiasis (OC) is one of the major opportunistic fungal infections that occur in over 90% of the HIV-infected individuals during the cause of their illness.[1] It is one of the Acquired Immune Deficiency Syndromes (AIDS) prognostic indicators and a major predisposing factor to invasive fungal diseases like blood-stream Candida infections.[2,3] Oral cavity colonization by *Candida* spp. in the healthy population ranges from 17%-75% worldwide.[4] For decades, *Candida albicans* has been the major *Candida* spp. isolated from oral cavity of both immunocompromised and immunocompetent individuals, accounting for 60%-80% of cases.[5,6] The emergence of non-*albicans Candida* (NAC) spp. which are more resistant to azoles causing oral candidiasis is of public health concern especially in resource constrained settings.[2,3] The common NAC spp. associated with oral candidiasis includes *C. glabrata, C. tropicalis, C. parapsilosis* and *C. krusei (Issatchenkia orientalis*).[7–9]

Oral candidiasis is among the indirect indicators for cell-mediated immunodeficiency and estimated to have >90% positive predictive value for invasive (e.g. blood stream) candidiasis.[4,10,11] Blood-stream *Candida* infections are the fourth-ranking hospital-related infection with mortality rates higher than bacterial blood-stream infections in USA.[5,12,13] With increased prevalence of immunocompromised patients in sub-Saharan Africa, there is widespread use of antifungal agents both for prophylaxis and treatment purposes.[9] This increased use has been associated with

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augmented reports of antifungal resistance.[14] There is increase of some yeast species like *I. orientalis, C. inconspicua* and *C. norvegenesis* which are intrinsically resistant to fluconazole being isolated from clinical specimens.[15] In addition, the increased use of antifungals especially azoles has resulted in multiple reports of fluconazole-resistant *C. glabrata*.[14,15]

In Tanzania, there are limited fungal diagnosis services available and data on *Candida* spp. colonization and subsequently causing infections in healthy individuals and immunocompromised patients are still scarce. This comparative study was designed to establish the prevalence and distributions of NAC spp. colonizing HIV-infected and non-HIV infected individuals.

2. Materials and Methods

2.1. Study design and cohort

This comparative cross-sectional study was conducted in Mwanza City, Tanzania between March and July 2015. Individuals aged 1- 90 years without clinical signs of oral candidiasis were recruited. Sample size was obtained using Kish Lisle formula basing on the 30% prevalence of NAC spp. obtained from a study in Nigeria among HIV-infected individuals.[16] The minimum sample size obtained was 323 HIV-infected individuals. In order to compare the prevalence we also enrolled nearly double as many non-HIV infected individuals as in the control group. HIV-infected patients (n=351) were recruited from Care and Treatment Centre (CTC) at Bugando Medical Centre (BMC). Non-HIV infected individuals (n = 639) were recruited from primary school, secondary schools, university and patients relatives visiting BMC. HIV status was confirmed using the Tanzania National Rapid test algorithms protocol.[17]

2.2. Data collection and processing

Social demographic data of the study participants were collected using standardized data collection tool. For HIV-infected patients, the most recent CD4+ count was obtained from files. Oral rinse specimens were collected by having each participant hold 10 ml sterile normal saline in the mouth for 60 seconds, then spit in the sterile container as previously described.[18] Samples were inoculated on Sabouraud's Dextrose Agar supplemented with 50 mg/ml gentamicin and 50 mg/ml chloramphenicol (SDA) (Oxoid, Basingstoke RG24 8PW, UK). Plates were aerobically incubated at 35 °C for 24-48 h. Preliminary spp. identification was done on CHROMagar (OXOID, Hampshire, England) as previously described.[19] The confirmation was done using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MALDI Biotyper, Bruker Daltonics, Bremen, Germany) on extracted cells harvested from SDA.[20,21]

3. Data analysis

Data analysis was done using STATA version 11. Categorical variables (sex, antibiotic use, use of HAART and trimethoprim/sulphamethaxazole (SXT) prophylaxes use) were summarized in the form of proportions and frequency tables, whereas median (interquartile range) was used to summarize continuous variables (age and CD4 count). Univariate and multivariate logistic regression analysis was done to determine factors associated with non-albicans Candida colonization. Factors with 95% confidence interval (CI) and p-value of less than 0.05 were considered statistically significant. The medians CD4 count among HIV infected individuals colonized and non-colonized with NAC spp. were compared using Wilcoxon rank-sum (Mann- Whitney) test.

3.1. Ethical consideration

Ethical clearance for conducting this study was granted by joint CUHAS/BMC research ethics and review committee with certificate number CREC/048/2014. All participants above 18 years of age were requested to sign the written informed consent form and for participants below 18 years their parents/guardians were requested to assents for them before recruitment. All patient-related information was stored in a pseudonymized form.

4. Results

The median age of HIV-infected patients was 38 (IQR 31-46) years while that of non-HIV infected individuals was 30 (IQR 26-36) years. The majority of HIV-infected patients (160/351, 45.6%) had a history of antibiotics use two weeks prior enrolment. The median CD4 count of the HIV-infected patients was 372.5 (IQR 195.5-559.5) cells/μl (Table 1).

Candida albicans was the commonest spp. detected in both groups 119 (n = 351, 33.90%) of HIV-infected and 146 (n = 639, 22.9%) of non-HIV infected population (p = 0.023). NAC spp. carriage was detected in 64 (n = 990, 6.46%) of the study participants. Of HIV-infected patients, 36/351 (10.3%) carried NAC spp., as compared to only 28/639 (4.4%) among non-HIV infected (P = 0.0003). Only one HIV infected participant was colonized by two NAC spp.

Of 351 HIV infected individuals; 10(2.8%), 9(2.6%) and 8(2.3%) had *Candida tropicalis*, *C. krusei* (*Issatschenki orientalis*) and *C. glabrata* respectively, while of 639 non-HIV infected individuals, *8*(1.3%) and 5(0.9%) were colonized with *C. dubliniensis* and *C. tropicalis* respectively (Table 2). Non-*albicans Candida* spp. detected from HIV infected individuals were *C. tropicalis* 10(27.8%), *I. orientalis* 9(25%), *C.glabrata* 8(22.2%) and others 9(25%) Table 2. Out of 15 *C. tropicalis*, 12 *I. orientalis* and 12 *C. glabrata* detected 10, 9 and 8 respectively, were from HIV infected

Table 1
Social demographic data of the study population

Variable	HIV positive (n = 351)	Control (n = 639)	P-value
Age (years)			
Median (IQR)	38(31-46)	30(26-36)	<0.001
Sex			
Female	262	462	
Male	89	177	0.426
Antibiotic use			
Yes	160(45.6%)	33(5.2%)	
No	191(54.4%)	606(94.8%)	<0.001
CD4			
Median(IQR)	372.5(195.5-559.5)	NA	NA
HAART			
Yes	343(97.2%)	NA	NA
SXT prophylaxis			
Yes	321(91.5%)	NA	NA

*SXT: trimethoprim/sulphamethaxazole

Table 2
Distributions of *Candi*da spp. among HIV and non HIV infected populations

Candida spp.	HIV positive N = 351, (n/N)%	Health control N = 639, (n/N)%	P value
C. albicans (265)	119(33.9)	146(22.9)	0.023
C. tropicalis (15)	10(2.8)	5 (0.9)	0.006
I. orientalis (12)	9 (2.6)	3 (0.5)	0.002
C. glabrata (12)	8 (2.3)	4 (0.6)	0.012
C. parapsilosis (11)	7 (1)	4 (0.6)	0.242
C. dubliniesis (11)	3 (0.8)	8 (1.3)	0.763
C. norvegensis (2)	0	2 (0.3)	NA
C. lusitaniae (2)	0	2 (0.3)	NA