HIV/*Candida* co-infection in Sub-Saharan African women on ART

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Abstract

Introduction: Sub-Saharan Africa has 23.5 million cases of HIV and is home to 92% of the world's HIV-positive pregnant women of whom 24% die of pregnancy related complications. Oral candidiasis is a common condition in HIV-AIDS patients, caused by commensal yeasts which may colonise the mucous membranes of the mouth causing morbidity due to several factors including immunosuppression, smoking, poor nutrition and the use of antibiotics.

Methods: One hundred and ninety-four South African and Cameroonian HIV-positive women participated in the study. Only subjects who had white pseudomembranous plaque on the tongue or visible oral candidiasis were included. Samples were collected by scraping the patient's oral mucosa and tongue with a sterile swab. *Candida* species were differentiated using selective and chromogenic media and their susceptibility to antifungal drugs was tested using the TREK Sensititre system.

Results and conclusion: One hundred and ninety-six isolates, representative of six *Candida* species were identified. *C. albicans* was the predominating species, with *C. glabrata* and *C. dubliniensis* being the more frequent of the non-albicans isolates. Azole drug resistance patterns were very high for *C. albicans*, while *C. glabrata* showed high resistance patterns to echinocandins drugs. The duration of ART could be associated with the presence of different *Candida* species but no concrete conclusions could be drawn concerning HIV/*Candida* co-infection when controlling for other risk factors such as HIV stage, pregnancy, age and treatment for tuberculosis. This may be a cause for concern, particularly in the case of pregnancy, where co-infection may pose a risk for maternal morbidity and mortality.

Introduction

One of the most common HIV-associated opportunistic infections is candidiasis, caused by the pseudohyphae-forming yeast of the *Candida* genus. These can cause an increase in patient morbidity and mortality due to oropharyngeal or systemic dissemination.

Candida is known to bind to the oral epithelial cells in women due to hormonal shifts during the menstrual cycle and the contraceptive pill. Seropositive women have been shown to have a higher oral colonization of *Candida* species, which increases with higher HIV viral loads. The oral colonization of *Candida* species in women has been reported to shift from *C. albicans* to non-albicans species over time and after antifungal therapy.

The introduction of highly active anti-retroviral therapy (HAART) did not greatly reduce the number of *Candida* infections over time, possibly due to antifungal prophylaxis over the years and consequent increase in non-albicans resistant species [1].

The colonization and resistance patterns of *Candida* species in HIV-positive women and their association with factors such as HAART, pregnancy, hormonal shifts, health status and drug resistance patterns have not been previously described. This deserves further investigation, as the presence of drug-resistant and non-albicans *Candida* species could seriously affect the wellbeing of these patients.

Objectives:

The objective of this study was to investigate the prevalence of *Candida* species in HIV-infected African females and to compare their colonization to susceptibility patterns and other parameters that may be distinguishing in women.

Materials and Methods

Approval from Ministry of Health Regional Hospital Institutional Review Board (IRB) in Cameroon and from the Ethics Committee at the University of the Western Cape was obtained. Patients presenting with white pseudomembranous plaque on the tongue or other visible oral candidiasis were selected. Sterile oral swabs were used to collect a sample from the affected areas in 194 HIV-positive women. Patients were required to sign consent forms and to submit some personal information in a questionnaire. Data from the patient's hospital file was also collected.

Swabs were plated onto Sabouraud's agar and grown at 30˚C on Fluka chromogenic *Candida* identification agar and Oxoid chromogenic *Candida* agar [2]. Confirmation of *Candida* species was achieved using microscopy, Gram staining and the germ tube test. Presumptive *C. albicans* and *C. dubliniensis* isolates were differentiated by growth at 37˚C for 48 hours in Tomato (V8) agar [3]; at 28˚C for 48-72 hours in Tobacco agar [4] and at 45˚C for 24-48 hours in Sabouraud dextrose agar [5]. Differences in growth, colony morphology and pseudohyphae/chlamydospore expression, allowed for species identification.

Drug susceptibility testing to azole (fluconazole, itraconazole and voriconazole) and echinocandin (anidulafungin, caspofungin and micafungin) drugs was done using the TREK Sensititre YeastOne 9 (YO9) system (Thermo Scientific, USA), a CLSI-approved broth micro-dilution method that allows for susceptibility testing of multiple antifungal drugs [6,7]. An isolate was considered to be resistant if it fell within the established "resistant" breakpoint category for one or more of the tested drugs in that class.

Statistical analysis for the species differentiation and susceptibility associations was done by Pearson Chi-square and Fisher's exact test using SPSS Version 21.0 (significant association: $p<0.05$).

Results

Out of the total 196 *Candida* strains isolated from the oral mucosa of 194 HIV-positive females, 152 were identified as *C. albicans*, 27 as *C. glabrata*, 8 as *C. dubliniensis*, 4 as *C. krusei*, 3 as *C. tropicalis* and 2 as either *C. parapsilopsis*, *C. lusitaneae* or *C. kefyr*. Two women were colonized by both *C. albicans* and *C. glabrata*.

Resistance to azole drugs was very high in the case of *C. albicans* (54%) and *C. krusei* (100%). High levels of resistance to echinocandin drugs was seen in the case of *C. glabrata* (40.7%), with only 2 (1.3%) of *C. albicans* isolates expressing resistance to this class of drugs.

No associations were seen between *Candida* species prevalence and age or health status. It was noted, however, that *C. albicans* was the only species isolated from the oral mucosa of patients who were either pregnant or had recently given birth.

Patients who had not yet started ARV medication were mostly colonized by *C. albicans*. However, a shift to non-albicans species was noted, as higher numbers of non-albicans species were seen in patients who were on ARV therapy for longer periods.

Table 1: Drug susceptibility and association results of *Candida* isolates.

	C. albicans	*C. dubliniensis*	*C. glabrata*	*C. tropicalis*	*C. krusei* *	*C. kefyr/para/lusi*	p-values
	(n=152)	(n=8)	(n=27)	(n=3)	(n=4)	(n=2)	
Age distribution							
10-20yrs (n=3)	3(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	
21-50yrs (n=163)	127(77.9%)	8(4.9%)	21(12.9%)	2(1.2%)	4(2.4%)	1(0.6%)	
>50yrs (n=30)	22(73.3%)	0(0%)	6(20%)	1(3.3%)	0(0%)	1(3.3%)	
Health status							
AIDS - (n=147)	112(76.2%)	8(5.4%)	20(13.6%)	2(1.4%)	3(2%)	2(1.4%)	
AIDS + (n=49)	40(81.6%)	0(0%)	7(14.3%)	1(2%)	1(2%)	0(0%)	
Pregnancy							
Not pregnant (n=182)	138(75.8%)	8(4.4%)	27(14.8%)	3(1.6%)	4(2.2%)	2(1.1%)	
Pregnant/recent birth (n=14)	14(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	
Patients on TB treatment							
No (n=172)	133(77.3%)	8(4.7%)	24(14%)	2(1.2%)	3(1.7%)	2(1.2%)	
Yes (n=24)	19(79.2%)	0(0%)	3(12.5%)	1(8.3%)	1(8.3%)	0(0%)	
ARV therapy							
No ARV therapy (n=35)	30(85.7%)	2(5.7%)	2(5.7%)	1(2.9%)	0(0%)	0(0%)	
AZT/NVP/3TC (n=73)	57(78.1%)	0(0%)	13(17.9%)	0(0%)	3(4.1%)	0(0%)	
d4T/NVP/3TC (n=31)	21(67.7%)	3(9.7%)	5(16.1%)	0(0%)	1(3.2%)	1(3.2%)	
d4T/EFV/3TC (n=24)	22(91.7%)	1(4.2%)	1(4.2%)	0(0%)	0(0%)	0(0%)	
AZT/EFV/3TC (n=17)	13(76.5%)	0(0%)	3(17.6%)	1(5.9%)	0(0%)	0(0%)	
LPV/r combinations (n=5)	3(60%)	1(20%)	1(20%)	0(0%)	0(0%)	0(0%)	
AZT/DDI/KLT (n=3)	1(33.3%)	1(33.3%)	1(33.3)	0(0%)	0(0%)	0(0%)	
TDF/3TC (n=2)	2(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	
AZT/3TC/lopinavir/ritonavir (n=1)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(100%)	
AZT/3TC/KLT (n=1)	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	
Not known (n=4)	2(50%)	0(0%)	1(25%)	1(25%)	0(0%)	0(0%)	
Duration of ARV therapy							
No ARV therapy (n=35)	30(85.7%)	2(5.7%)	2(5.7%)	1(2.9%)	0(0%)	0(0%)	p=0.008
<1yr (n=50)	43(86%)	1(2%)	6(12%)	0(0%)	0(0%)	0(0%)	
≥1-<3yrs (n=49)	35(71.4%)	5(10.2%)	7(14%)	1(2%)	1(2%)	0(0%)	
≥3yrs (n=60)	44(73.3%)	0(0%)	11(18.3%)	0(0%)	3(5%)	2(3.3%)	
Unknown (n=2)	0(0%)	0(0%)	1(50%)	1(50%)	0(0%)	0(0%)	
Susceptibility patterns	Susceptible / Resistant	Susceptible / Resistant	Susceptible / Resistant	Susceptible / Resistant	Susceptible / Resistant	Susceptible / Resistant	
Azoles	70(46%) / 82(54%)	7(87.5%) / 1(12.5%)	22(81.5%) / 5(18.5%)	3(100% / 0(0%)	0(0%) / 3(100%)	2(100%) / 0(0%)	p=0.000
Echinocandins	150(98.7%) / 2(1.3%)	- / -	16(59.3%) / 11(40.7%)	3(100%) / 0(0%)	3(100%) / 0(0%)	- / -	p=0.000

*One of the *C. krusei* isolates did not grow on the TREK plate, and was therefore not included in the susceptibility section of this table.
"-" = no established breakpoint for the organism/drug.

Discussion

The high levels of antifungal drug resistance seen in this cohort of women is worrying and deserves further attention, especially since the drug of choice for *Candida* infections in the African continent (fluconazole) was found to be ineffective against a large amount of clinical *Candida* isolates. This is also a cause of concern due to the fact that only albicans species (which expressed the highest resistance levels to azoles) were found in pregnant mothers, which could pose a risk for pre-term delivery and maternal morbidity and mortality [8].

The shift from *C. albicans* to the more drug-resistant non-albicans species seen after continued ART is also a cause of concern that has not been previously documented. We propose better monitoring of these patients, as the emergence on non-albicans species and the empirical dispensation of antifungal drugs in resource-limited healthcare facilities might not be effective in treating *Candida* infections in these populations.

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