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Impact of Eating Probiotic Yogurt on Colonization by *Candida* Species of the Oral and Vaginal Mucosa in HIV-Infected and HIV-Uninfected Women

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Abstract

Background—Candidiasis in HIV/AIDS patients continues to be a public health problem. Antifungal therapies are not always effective and may result in complications, such as the development of drug-resistant strains of *Candida* species.

Objectives—This study evaluated the impact of probiotic consumption on *Candida* colonization of the oral and vaginal mucosa.

Patients/Methods—A pilot study was conducted in 24 women (17 HIV-infected, 7 HIV-uninfected) from the Women's Interagency HIV Study. The women underwent a 60-day initiation period with no probiotic consumption, followed by two 15-day consumption periods, with a

different probiotic yogurt (DanActive™ or YoPlus™ yogurt) during each interval. There was a 30-day washout period between the two yogurt consumption periods. Oral and vaginal culture swabs were collected on days 0, 60, 74, and 120. *Candida* was detected by inoculating each swab in both Sabouraud's dextrose agar with or without chloramphenicol and CHROMagar.

Results—Less fungal colonization among women was observed when the women consumed probiotic yogurts (54 % of the women had vaginal fungal colonization during the non-probiotic yogurt consumption period, 29 % during the DanActive™ period, and 38 % during YoPlus™ yogurt consumption period), and HIV-infected women had significantly lower vaginal fungal colonization after they consumed DanActive™ yogurt compared to the nonintervention periods (54 vs 29 %, $p = 0.03$).

Conclusions—These data are promising, but as expected in a small pilot study, there were some significant changes but also some areas where colonization was not changed. This type of conflicting data is supportive of the need for a larger trial to further elucidate the role of probiotic yogurts in fungal growth in HIV-infected women.

Keywords

Probiotics; Vulvovaginal candidiasis; Oral candidiasis; *Candida*; HIV; Opportunistic infections

Introduction

Mucosal candidiasis, which includes oropharyngeal (thrush), esophageal, and vaginal candidiasis, is common among people infected with HIV [1]. Vulvovaginal candidiasis (VVC) is one of the most common infections in all women; however, it occurs even more frequently in HIV-infected women [1, 2]. The annual cost of VVC in the USA in 1995 was \$1.8 billion and is expected to rise to \$3.1 billion in 2014 [2]. Oral candidiasis disproportionately impacts HIV-infected patients, affecting up to 90 % of AIDS patients [3, 4]. Various topical and systemic treatments are recommended to treat candidiasis; however, many of these treatments have produced drug-resistant strains of the fungus [5].

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [6]. Yogurts and probiotics are commonly used by women when they suspect they have a vaginal infection. However, the evidence for their effectiveness is limited [7, 8]. Several studies have discussed using probiotics as an alternative treatment for candidiasis [7, 9, 10]. A survey carried out by Pirodda et al. [11] found that among 1,117 women between the ages 18 and 70, 73 % self-reported symptoms of VVC in the past, while 40 and 43 % of these women reported that they used *Lactobacillus* products for prevention and treatment for post-antibiotic vulvovaginitis, respectively. Some studies suggest that some probiotics can inhibit the adherence and/or the growth of *C. albicans*, while others have demonstrated that the lack of lactobacilli species that can produce H₂O₂ was associated with increase in VVC [12, 13]. Hilton et al. [14] found a threefold decrease in vaginal candidal infections and a significant decrease in mean candidal colonization among women who daily ingested eight ounces of yogurt containing *Lactobacillus acidophilus* versus those women in the control group. Martinez et al. [9] found that probiotics *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 combined with fluconazole augmented the cure rate of yeast vaginitis. Zwolinska-Wcislo et al. indicated that probiotics therapy had effective treatment for fungal colonization of gastrointestinal tract [15]. In this study, a lower presence of fungal colonization was observed among study women when they consumed probiotic yogurts, and a statistically significant reduction in vaginal fungal colonization was found after women consumed DanActive™.

The majority of yogurts on the market are not supplemented with additional well-studied probiotic strains. Of the few that are well studied, these two yogurts were selected for the study to represent the two major genera, *Bifidobacterium* and *Lactobacillus*. This study aimed to identify whether eating yogurt containing high-dose, well-studied probiotic strains would benefit women by reducing their vaginal and oral colonization by the *Candida* species.

Subjects and Methods

Study Population

Women for this study were recruited from the Women's Interagency HIV Study (WIHS), an ongoing longitudinal study evaluating HIV prognosis and related conditions in women previously described in the literature [16, 17]. Twenty-five participants were recruited; 18 were HIV-infected and 7 were HIV-uninfected. One participant who was HIV-infected subsequently withdrew from the study and 24 women remained in the study.

Two-thirds of the women in the WIHS cohort are HIV-infected. Women were excluded from this study if they had hysterectomies, were trying to get pregnant, or were treated with any antifungal agent during initiation of the study. Women were prospectively followed, and oral and vaginal samples were collected at four separate times over a 4-month period. Written informed consent was obtained from each participant, and the study was approved by the Georgetown University Institutional Review Board prior to study commencement.

Data Collection

The participants were visited on days 0, 60, 74, and 120; in these visits, oral and vaginal culture swabs were collected and a questionnaire was completed. On day 60, the women were provided with a 15-day supply of DanActive™ and were instructed to consume one 3.1-ounce yogurt per day. The women kept a diary of the date and time that the yogurt was consumed, as well as any symptoms experienced during consumption. The participants then underwent a 30-day washout period, in which they were instructed not to eat any yogurt or probiotic products. On day 106, they were given a 15-day supply of YoPlus™ and instructed to eat one 4-ounce yogurt per day, keeping the same diary as during the DanActive™ consumption period.

Fungal Identification

Direct microscopy (10 % KOH) of swabs is effective to detect fungal morphological forms (budding yeast or hyphae) in smears when the organism presents in high numbers and is therefore most probably causing disease. However, the species identification requires other selective media or biochemical assays. Therefore, each swab was inoculated in both Sabouraud's dextrose agar with (SABC) or without chloramphenicol (SAB), as well as CHROMagar. Since some *Candida* species do not grow on the SABC medium, SAB ensured the optimal fungal recovery. CHROMagar-*Candida* is useful in differentiating among species of *Candida* since they produce different colored colonies, especially *C. albicans* and *C. glabrata*, which together account for the majority of all *Candida* isolates [18]. Germ tube and chlamyospore assays were setup for *C. albicans* only. In the germ tube assays, yeast was inoculated in 0.5 mL of 10 % sera and incubated aerobically at 37 °C for 2 h. A drop of the yeast-serum mixture was examined microscopically, and the appearance of small filaments projecting from the yeast cell surface indicates that the germ tube was positive. In the chlamyospore assays, yeast cells were plated onto cornmeal agar under a glass coverslip to maintain a semi-anaerobic condition and grown in the dark for 7 days at 25 °C. Plates were examined over the following 21 days for chlamyospores. The remaining

undetermined strains were tested on API 20C system (BioMerieux) [19] to assign a species. All the isolates were stored at -80°C for future fluconazole susceptibility tests [15, 20].

Outcome Variables

The presence of vaginal and oral fungal colonization among participants was the primary outcome of the study; this was coded as present or not present. Persistent mucosal *Candida* colonization was defined as the presence of organisms two or more times during the study period. Other self-reported symptoms and conditions such as the presence of constipation, loose stool, vaginal infections, use of over-the-counter (OTC) medicines, and diarrhea in the past 30 days were collected as the secondary outcomes and were also coded as present or not present.

Statistical Analysis

The pilot study consisted of 24 women who were followed for 120 days. An exact McNemar's test was fitted to determine whether there were differences in the presence of vaginal/oral fungal colonization among participants during the following three study periods: 1) the non-probiotic consumption period, 2) the DanActive™ yogurt consumption period, and 3) the YoPlus™ yogurt consumption period. Other health conditions of the women, such as experiencing loose stools, vaginal infection, and/or constipation during last 30 days of the study, were also compared during these three study periods. SAS 9.2 (SAS Institute Inc., Cary, NC, USA) was used for all the statistical analysis.

Results

The characteristics of the women in the study compared to the WIHS cohort are shown in Table 1. There were more African-American women and fewer Hispanic participants in the study than in the entire WIHS cohort. The study population also had higher viral loads.

Primary Outcome

Vaginal Candidiasis—More HIV-infected women had vaginal *Candida* colonization than the HIV-uninfected women (77 vs 43 %). *C. albicans* (77 % in HIV-infected women and 100 % in HIV-uninfected women) was the most common mucosal species identified among the women who had vaginal colonization (Table 2). An exact McNemar's test among all the 24 study women (Table 3) indicated that presence of vaginal fungal colonizations was different during the three study periods. Approximately 54 % ($n = 13$) of the women had vaginal fungal colonization during the non-probiotic yogurt consumption period, 29 % ($n = 7$) of the women had vaginal fungal colonization during the DanActive™ yogurt consumption period, and 38 % ($n = 9$) of the women had vaginal fungal colonization during the YoPlus™ yogurt consumption period. The result for the DanActive™ yogurt consumption period was statistically significant compared to the non-consumption periods ($p = 0.03$). No statistically significant differences in vaginal fungal colonization presence were found between the two probiotic yogurt periods or between the non-probiotic yogurt period and the YoPlus™ yogurt period.

The participants were categorized by their HIV status, and there was a lower presence of vaginal fungal colonization among both HIV-infected and HIV-uninfected participants during the probiotic yogurt consumption periods (Fig. 1). However, due to sample size, these differences were not significant.

Oral Candidiasis—Both HIV-infected and HIV-uninfected women had surprisingly high rates (71 %) of oral candidal colonization in the study. Similar to that of vaginal colonization, *C. albicans* was the most identified species in oral candidal colonization (83 %

in HIV-infected women and 80 % in HIV-uninfected women) (Table 2). There were no statistically significant differences of oral fungal colonization among all the study women during the different study periods in the exact McNemar's test (Table 3). The percentage of the women who had oral fungal colonization were 63 % ($n = 15$) during the non-probiotic yogurt period, 50 % ($n = 12$) during the DanActive™ yogurt period, and 58 % ($n = 14$) during the YoPlus™ yogurt period. When the participants were grouped by their HIV status, Fig. 1, we did not observe a lower presence of oral fungal colonization among HIV-uninfected women during the study period. A lower presence of oral fungal colonization was observed in HIV-infected women when they ate probiotic yogurts, most impressively during the DanActive™ yogurt consumption period. However, this was not statistically significant due to the small sample size.

Secondary Outcomes

Fewer women (statistically significant) used OTC medicine while taking probiotic yogurts compared with when they consumed non-probiotic yogurt (Table 3). The percentage of the women who used OTCs were 78 % during the non-probiotic yogurt period, 42 % during the DanActive™ period, and 46 % during the YoPlus™ period ($p = 0.01$: non-probiotic yogurt period versus DanActive™ period; $p = 0.02$: non-probiotic yogurt period versus YoPlus™ yogurt period). Fewer women (statistically significant) also had constipation during the probiotic yogurt periods than during the non-probiotic yogurt period. The percentage of the women who had constipation were 58 % during the non-probiotic yogurt period, 29 % during the DanActive™ period, and 21 % during the YoPlus™ period ($p = 0.02$: non-probiotic yogurt period vs DanActive™ period; $p < 0.01$: non-probiotic yogurt period versus YoPlus™ period) (Table 3). There were no changes in the fungal species for the women after probiotic treatment for any of the yogurt products consumed.

Discussion

The presence of vaginal colonization was much higher among HIV-infected women in this study than HIV-uninfected women (77 vs 43 %). Persistent vaginal colonization presence was also much higher in HIV-infected women than in HIV-uninfected women (41 vs 29 %). These findings are consistent with other studies [1]. The high rates of oral fungal presence in all the participants, 71 % in both HIV-infected and HIV-uninfected women, were a particularly surprising and potentially very important finding. The potential for these organisms to become pathogenic is significant and important, as the treatment options are limited. Among the women who had positive colonization, *C. albicans* was the most common species that was found in both HIV-infected and HIV-uninfected women on both oral and vaginal sites, and this result is consistent with other studies [9, 21].

Another theory is that probiotics could benefit candidiasis colonization among HIV-infected women with an increase in CD4 count of people living with HIV/AIDS [7, 22–24]. Some studies indicated that oral candidiasis occurred with increasing frequency as the CD4 count dropped below 500 cells/mm³ [1, 25]. Hummelen et al. [26] demonstrated that higher CD4 counts lead to lower vaginal fungal growth. Therefore, some researchers have recommended yogurts that contain probiotic products as a potential prevention and treatment that would benefit the immune system of HIV-infected people. A significant drop in the use of OTCs in the past 30 days was found in the women in this study during both probiotic yogurt consumption periods compared with that of non-probiotic yogurt consumption periods. This could be an indirect sign of the improvement of immune systems among the study women. There was also a significant reduction in constipation occurrence among the study women when they consumed probiotic yogurts compared with when they did not consume probiotic yogurt. This result is consistent with other studies [27, 28].

This study is one of the few studies that examined the effect of probiotic yogurt consumption and fungal colonization condition in HIV-infected women [24]. Significant limitations of this study were the sample size and the relatively short follow-up period; however, this is common in a pilot study such as this in which limited information exists. Due to the small sample size, it was not possible to adjust or examine other important covariates related to fungal colonization. A lower fungal presence among the study women was observed after they consumed probiotic yogurts, 29–38 % of the women experienced fungal colonization, compared with 54 % during the non-probiotic yogurt consuming period; however, only a statistically significant reduction in vaginal fungal colonization after women ate DanActive™ was observed. For convenience, all women started with DanActive™ and then proceeded with YoPlus™. Although there was a 30-day washout period between the two consumption periods, it is possible that the more positive impact observed with DanActive™ was due to the women having a higher likelihood of colonization. Future studies should vary the starting probiotic or conduct different large randomized trials where women only consume one product during the study.

Since this is a pilot study, two different probiotic yogurts that cover the two major genera were included. Different effects among these strains should be tested in the future. These results indicate that consumption of a probiotic yogurt could reduce fungal colonization and some symptoms in HIV-infected and HIV-uninfected women. Therefore, eating probiotic yogurt could offer a safe and well-tolerated alternative, supplemental treatment of HIV-infected women to improve their quality of life, especially when many of the various topical and systemic treatments have produced drug-resistant strains. This is promising and supportive of the need for a larger trial to further elucidate the role of probiotic yogurts in fungal growth in HIV-infected women.

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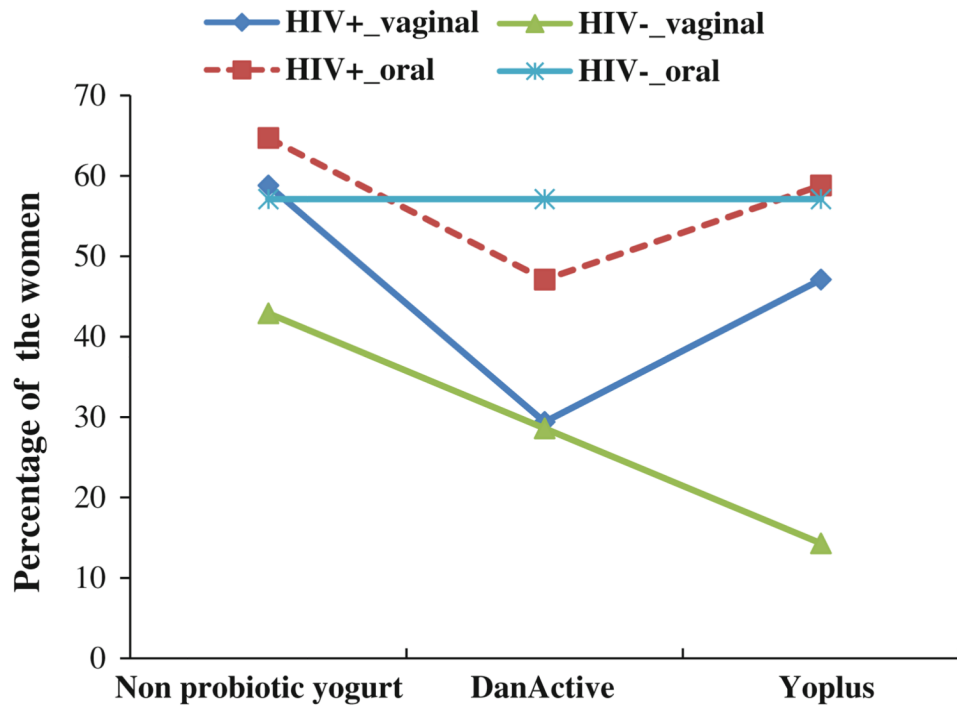


Fig. 1. Percentage of women with *candida* colonization during the different study period by HIV status

Table 1
Characteristics of study women and all WIHS women in visit 32

Characteristics	Study participants (N=24)	All WIHS participants (N=1,901)
Race (%) [#]		
Other	4.17	3.68
Hispanic	4.17	27.51
Black (non-hispanic)	87.50	56.18
White(non-hispanic)	4.17	12.62
Income(\$12,000/year %)	50.00	53.15
Depression (%)	25.00	30.02
Education (%)		
Above high school	29.17	33.19
High school	45.83	29.97
Less than high school	25.00	36.83
CD4 (%)		
<35	20.83	25.25
350 and 500	8.33	13.73
>500	70.83	71.60
HIV+(%)	70.83	71.60
HAART use ^a (%)	76.47	84.13
Log viral load ^a (mean) [#]	6.48	5.42

^a HIV+only

[#] Statistical significant

Table 2
Vaginal/oral candidal colonization among study women by HIV status

Outcome	HIV positive N (%)	HIV negative N (%)
Vaginal candidal colonization	13 (77)	3 (43)
Persistent vaginal candidal colonization	7 (41)	2 (29)
Oral candidal colonization	12 (71)	5 (71)
Persistent oral candidal colonization	11 (65)	3 (43)
Vaginal candidiasis species identified		
<i>C. albicans</i>	10 (77)	3 (100)
<i>C. glabrata</i>	2 (15)	
<i>C. sphaerica</i>	1 (8)	
Oral candidiasis species identified		
<i>C. albicans</i>	10 (83)	4 (80)
<i>C. glabrata</i>	3 (25)	
<i>C. lusitanae</i>	1 (8)	
Kloeckero		1 (20)

Table 3
McNemar test results of vaginal/oral candidal colonization and other symptoms among study participants

Characteristics	Non-probiotic yogurt period <i>n</i> (%)	Danactive™ period <i>n</i> (%)	Yoplus™ period <i>n</i> (%)
Had loose stool in past 30 days	10 (41.67)	5 (20.83)	8 (33.33)
Use over the counter medicine in past 30 days	19 (78.17)	10 (41.67)*	11 (45.83)#
Had diarrhea in past 30 days	9 (37.50)	6 (25.00)	6 (25.00)
Had constipation in past 30 days	14 (58.33)	7 (29.17)*	5 (20.83)#
Had vaginal infection in past 30 days.	2 (8.33)	2 (8.33)	3 (12.50)
Vaginal fungi growth	13 (54.17)	7 (29.17)*	9 (37.5)
Oral fungi growth	15 (62.5)	12 (50)	14 (58.33)

* $P < 0.05$ compared no special yogurt consumption period and DanActive™ consumption period

$P < 0.05$ compared no special yogurt consumption period and Yoplus™ consumption period