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THE PREVENTIVE EFFECT OF PROBIOTICS CONCERNING NEW DENTURE WEARERS IN ORAL CANDIDIASIS

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ABSTRACT

INTRODUCTION: The most common therapeutic strategy for oral candidiasis is using antifungal agents, Unfortunately, the prolonged administration of antifungals has many adverse effects. There is an increasing demand for a potent antifungal agent against oral candidiasis (OC) with the least adverse effect and low recurrence rate.

OBJECTIVES: To evaluate the preventive effect of a daily dose of commercial probiotics on the oral Candida spp. colonization in new denture wearers during probiotics administration and 4 weeks after the end of probiotics intake.

MATERIAL AND METHODS: The current clinical trial included 48 new complete denture wearers with detectable levels of *Candida* but without clinical symptoms. Participants in the probiotics group received a daily dose of probiotics lozenges for eight weeks versus the placebo tablets taken by the participants in the placebo group. Microbiological mouth-rinse samples determined the *Candida* count at different time intervals: baseline, two weeks after denture delivery, after 4 and 8 weeks of intervention, and four weeks after post-intervention follow-up.

RESULTS: Probiotics significantly reduced *Candida* count compared to placebo in the fourth week and subsequent follow-up periods. In the eighth week, they had the highest decrease ($p < 0.0001$).

CONCLUSION: Probiotic lozenges were highly effective against fungal infections in new denture wearers, with short-term preventive effects even after intervention cessation.

KEYWORDS: Probiotics, Candidiasis, New denture wearers, Denture stomatitis

RUNNING TITLES: Prophylaxis effect of probiotics for oral candidiasis.

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INTRODUCTION

Oral *Candida* infection is prevalent among elderly people, especially denture wearers. The prevalence of this infection can range from 15% to 70% (1,2). This might be due to systemic causes such as diabetes, prolonged antibiotic treatment, polypharmacy, hypo-salivation, immune deficiencies, or local factors such as inadequate oral hygiene and denture wearers (3).

Candida species are commonly found in the oral cavity as harmless microorganisms. *Candida albicans* is the most predominant and consequential yeast species that becomes pathogenic when dysbiosis in the oral biofilm occurs. The pathognomonic *Candida* array ranges from inflammatory to premalignant conditions (4,5).

The most common treatment for oral thrush is an antifungal medication, such as nystatin, miconazole, and fluconazole, in combination with oral hygiene measurements (5). Regrettably, prolonged use of antifungal medications has many side effects such as *Candida* developing resistance to antifungal agents, dysgeusia, and GIT problems (6). Additionally, the use of antifungal treatments often results in a high rate of recurrence and early relapse following the completion of treatment (7). Hence, preventive and therapeutic alternatives for oral candidiasis that do not rely on antifungal medications were investigated (8).

Probiotics are live microorganisms that, when administered in adequate amounts, can confer health benefits on the host (9). Oral candidiasis is a result of a disorder in the oral biofilm, which makes probiotics an interesting bioecological approach for the prevention and management of this condition. This is because probiotics have competitive, antagonistic, and immunological effects against pathogenic microorganisms (10).

This study aims to inspect the effect of using a daily dose of commercial probiotics containing 4 lactobacilli strains (*Lactobacillus. salivarius, Lactobacillus. reuteri, Lactobacillus. paracasei,* and *Lactobacillus. sakei*) on the Oral of *Candida* colonization in new denture wearers. Also, this study aims to evaluate the relapse in oral *Candida* colonization levels after 4 weeks of cessation of probiotics intake.

The null hypothesis of our study is that using a daily dose of probiotics would not affect the *Candida* colonization level in the oral cavity.

SUBJECTS AND METHODS

Patients' selection

We initially examined one hundred volunteers who attended the outpatient clinics to fabricate new complete dentures in the Faculty of Dentistry, University of Alexandria, of whom 48 were eligible to participate in the present study.

Informed consent

All participating patients were provided with detailed information about the intervention and given their consent. The Ethics Committee of the Faculty of Dentistry, Alexandria University, approved the study.

Sample size estimation

The sample size was estimated assuming a 5% alpha error and 80% study power. According to Passariello et al., (11), the mean percent reduction in the *Candida* albicans count after using probiotic preparation was calculated to be 0.743, and the level of significance 95% (α=0.05), the minimum sample size was calculated to be 24 patients per group. Total sample size = Number per group x Number of groups = 24 x 2 = 48 patients.

Inclusion criteria

Detectable *Candida* colonization in oral mucosa without any symptoms of candidiasis.

Patients with newly formed dentures in both arches.

The study participants included individuals with controlled hypertension and diabetes.

Exclusion criteria

Inability to understand/ follow the experimental procedures.

The participants who received topic or systemic antifungal or antibacterial agents in the previous 60 days.

Utilization of probiotics.

GIT problems, and cardiovascular disease.

Immune system diseases.

Oral thrush symptoms.

Intervention

a) Study group A

Participants were examined clinically and microbiologically during denture fabrication as a negative control. After denture insertion, baseline samples were collected. Participants took daily probiotic lozenges for 8 weeks, with samples collected every 4 weeks. The probiotic tablets containing 10^9 CFU of *lactobacilli* strains (*L. salivarius, L. reuteri, L. paracasei*, and *L. sakei*) per tablet with berry flavor were commercially available from (Nuveda Wellness, Canada).

b) Control group (group B)

24 participants received a lozenge placebo this product, prepared in the Faculty of Pharmacy, Alexandria University, had the same characteristics as the probiotic product (shape, size, color, and texture) but without the probiotic bacteria. Both lozenge tablets were 4°C refrigerated until their handling with participants. All participants received identical packages for both probiotics and placebo.

Microbiology analysis

Collect a sample of oral rinse solution. The mouth was rinsed with 10 mL of phosphate buffer saline (PBS), which was held in the mouth for 60 seconds. The sample was then promptly transported to the microbiology laboratory. For culturing and isolation of *Candida* colonization, continuous rinse specimens were vortexed, and centrifuged at 10,000 x g at 4° C for 5 min, then 10 ml and100 mL were directly streaked on the surface of prepared plated media composed of Sabouraud dextrose agar (SDA) *Candida* medium mixed with chloramphenicol (0.1 mg/mL) to control bacterial overgrowth.

Following a 48-hour incubation period at a temperature of 37°C, the measurement obtained was the number of colony-forming units per milliliter of saliva (CFU/mL) by the equation: CFU/mL = n° of colonies × dilution factor.

Statistical analysis of data

Data was analyzed using IBM SPSS version 23. Normality was checked using the Shapiro-Wilk test and Q-Q plots, which revealed a non-normal distribution. Therefore, median, minimum, and maximum values were used in addition to mean and standard deviation for data presentation. Qualitative data was presented using frequency and percentage. Percent change in Log10 fungal count was calculated as follows: [(Follow up values – Baseline value)/Baseline value]x100. Age was compared using an independent t-test. Pearson Chi-square or Fisher's test was used to compare qualitative variables between groups. Log10 fungal count was compared using the Mann-Whitney U test, while the Freidman test with Bonferroni correction was performed to assess differences across time points within groups. All tests were two-tailed (p value≤0.05).

RESULTS

Demographic distribution of the study groups

Forty-eight individuals who wore dentures and had measurable levels of Candida colonization without noticeable symptoms were divided into two equal groups: Group A (receiving probiotics) and Group B (receiving a placebo).

In the probiotics group, the age of patients (n=24) had a mean±SD. of 60.20±9.80 years, while in placebo (n=24) had a mean±SD. of 61.45±9.70 There was no statistically significant difference in the age between the two studied groups (*p*=.655)

Individuals assigned to the placebo or probiotic group showed no disparities in social factors, including age, and gender. Additionally, other attributes such as smoking, medical conditions, and denture hygiene were also similar between the groups. (Table 1)

Candida count assessment

At baseline, all participants harbored *Candida*, and there were no differences in *Candida* levels between the study groups (probiotics) and the control group (placebo) (*p*=.808).

After four weeks of the intervention, it was observed that the count of *Candida* was statistically significantly decreased in the probiotics group compared to the placebo group (p<.0001). In the probiotics group, the level of *Candida* colonization ranged from 1.00 to 3.30, with a median of 2.00. On the other hand, in the placebo group, the level of *Candida* infection ranged from 2.30 to 3.48, with a median of 2.91.

On comparing the mean between the two study groups in eight weeks, there was a statistically significant difference (*p*<.0001) In the probiotics group, the log level of *Candida* level ranged from 0.78 to 1.01, with a median of 0.00. In Group B, the log level of *Candida* level ranged from 2.30 to 3.57, with a median of 3.00.

The count level of *Candida* colony in the probiotics group was significantly reduced compared to the placebo group after four weeks of cessation. (p < 0.0001) (Table 2)

Table 1: The demographic, clinical, and medical parameters of the participants.

		Probiotics (n=24)	Placebo (n=24)	*p-value*
Age: Mean ± SD		60.20±9.80	61.45±9.70	0.655ǂ
Gender: n (%)	Females	7 (32%)	7 (32%)	1.00†
	Males	17 (68%)	17 (68%)	
Medical condition: n (%)	No	12 (52%)	13 (48%)	1.00†
	Yes	12 (47%)	11 (51%)	
Type of medical condition: n (%)	Diabetes	7 (74%)	7 (68.2%)	1.00†
	Hypertension	1 (16.7%)	1 (15.4%)	
	Both	1 (8.3%)	1 (15.4%)	
Smoking: n (%)	No	14 (56%)	14 (56%)	1.00†
	Yes	10 (44%)	10 (44%)	
Oral hygiene: n (%)	Good	11 (48%)	10 (48%)	1.00†
	Poor	13 (52%)	14 (52%)	

ǂ: Independent t-test, **†:** Pearson Chi Square test

Table 2: The difference in *Candida* count between the probiotics and placebo during different intervals.

		Probiotics (n=24)		Placebo (n=24)		*p* value¥
		Mean ± SD	Median (Min-Max)	Mean ± SD	Median (Min-Max)	
Baseline (Before denture)	Count	$1.04\pm1.06\times10^3$	5.50×10^3 (3.00×10 – 4.00×10^3)	$7.21\pm5.30\times10^2$	6.20×10^2 (1.20×10^2 – 2.50×10^3)	0.808
	Log	2.77±0.54	2.74 (1.48 – 3.60)	2.77±0.29	2.79 (2.08 – 3.40)	
2 Weeks (After denture)	Count	$1.23\pm1.08\text{x}10^3$	$8.00\text{x}10^2$ ($4.50\text{x}10$ – $4.10\text{x}10^3$)	$8.17\pm5.74\text{x}10^2$	$7.00\text{x}10^2$ ($2.00\text{x}10^2$ – $2.75\text{x}10^3$)	0.299
	Log	2.89±0.50	2.90 (1.65-3.61)	2.83±0.26	2.85 (2.30-3.44)	
4 Weeks (After Rx)	Count	$3.24\pm5.44\times10^2$	1.00×10^2 (1.00×10 – 2.00×10^3)	$8.69\pm5.91\times10^2$	8.20×10^2 (2.00×10^2 – 3.00×10^3)	<0.0001*
	Log	2.01±0.70	2.00 (1.00 – 3.30)	2.85±0.29	2.91 (2.30 – 3.48)	
8 Weeks (After Rx)	Count	5.00±8.35×10	0.00 ($0.00 – 2.80\times10^2$)	$1.30\times10^3\pm8.50\times10^2$	1.00×10^3 (2.00×10^2 – 3.70×10^3)	<0.0001*
	Log	0.78±1.01	0.00 (0.00 – 2.45)	3.00±0.35	3.00 (2.30 – 3.57)	
12 Weeks (After Rx)	Count	$3.18\pm3.24\times10^2$	2.50×10^2 ($0.00 – 1.35\times10^3$)	$2.30\pm1.41\times10^3$	2.00×10^3 (1.30×10^2 – 5.70×10^3)	<0.0001*
	Log	2.11±0.88	2.40 (0.00 – 3.13)	3.25±0.37	3.30 (2.11 – 3.76)	
p value#		<0.0001*		<0.0001*		

*Statistically significant difference at *p* value≤0.05, **¥:** Mann Whitney U test, **#:** Friedman test

DISCUSSION

Changes in the oral microbiota in denture wearers are the main cause of (OC) (12,13). There is increasing demand to find potent therapeutic and prophylaxis antifungal agents with less harmful effects (14). Thus, this study was a clinical trial to assess the preventive effect of blended multi-strain probiotics as a potential preventive (OC) conducted on 48 patients having a new denture.

There have been few studies on the effectiveness of probiotics against oral *Candida* in patients who do not show symptoms. Most studies have focused on patients with oral candidiasis, and probiotics have been used as an adjuvant to conventional therapy rather than the sole treatment to reduce *Candida.* A recent study showed that using a probiotic product consisting of *Lactobacillus bulgaris, Bifidobacterium longum,* and *S. thermophilus* along with oral local antifungal agents (nystatin) was more effective in treating *Candida*-associated stomatitis than conventional therapy (15).

According to a study conducted by Ishikawa, asymptomatic elderly denture wearers who used a probiotic product containing *L. acidophilus, L. rhamnosus,* and *Bifidobacterium bifidum,* have demonstrated a reduction in oral *Candida* prevalence.

Similarly, the present study demonstrated that using a probiotic product with *L. salivarius, L. paracasei, L. reuteri, L. paracasei, and L. sakei* was effective in significantly decreasing the *Candida* load in new asymptomatic denture wearers after four and eight weeks. Our study reveals that the cocktail of probiotics can prevent oral thrush in new denture wearers. In a similar study conducted by Miyazima et al., (16), it was observed that daily consumption of cheese supplemented with probiotics containing either *L. acidophilus* or *L. rhamnosus* could decrease the colonization of oral *Candida* in complete denture wearers. This highlights the potential benefits of probiotics in preventing oral candidiasis in this highly susceptible population.

The study used a systematic approach consistent with previous research. Probiotics were given in cheese to evaluate their potential to reduce the risk of *Candida* infection in high-risk populations (17, 18). They were also given as other dairy products to examine the effect of probiotics on *Candida* colonization in different populations

(19, 20). In this study, probiotics were provided in the form of lozenges for ease of storage, administration, and availability, in addition to benefit from a combination of four probiotic strains.

In our study, after four weeks of cessation of treatment, the probiotics cocktail demonstrated a reduction in *Candida* load moreover this cocktail provides a short-term effect to prevent oral candidiasis in new denture wearers. In contrast, in oral lichen planus coinfected with *Candida*, probiotic lozenges containing two strains of *L. reuteri* showed no significant difference in *Candida* counts between study groups after long-term follow-up, attributed to the significant dropping out of participants during the study.

Our study can be considered the first study to investigate the short-term effect after cessation of four blended probiotics *L. salivarius, L. reuteri, L. paracasei, and L. sakei* which has not been reported before in previous studies on asymptomatic new denture wearers. Furthermore, the blended probiotics reveal effectiveness in preventing OC.

From our point of view, the limitations of this study are the small sample size and the short-term follow-up period.

CONCLUSION

Based on the findings, we concluded the following:

Lozenge probiotics reduce the number of candida colonies.

Probiotics with blended multi-strains are used as a prophylaxis treatment to prevent OC for new denture wearers.

RECOMMENDATION

Future research should confirm the changes in denture colonization with larger samples and long-term probiotic effects.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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