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ORIGINAL ARTICLE

Effect of Probiotics on *Candidal* Adherence on Different Denture Base Resins in Maxillofacial Patients

[1]Akash Verma, [2]Sunil Kumar, [3]Harikesh Rao

ABSTRACT

The study was undertaken with the objective of evaluating the effect of probiotics on *Candidal* adherence on different heat cure denture base materials in maxillofacial patients. After Acralyn-H, Pyrax heat cure denture base resin showed less adherence to heat followed by Dental Products of India (DPI) heat cure denture base resin. *Candidal* cell count was significantly reduced on different heat cure denture base materials after giving probiotics to maxillofacial patients. The heat cure polymethylmethacrylate (PMMA) specimens were fabricated respectively, 60 samples in total, 30 samples before giving probiotics, and 30 samples after giving probiotics. The adherence of yeast on the tissue surface of the specimens was given prime consideration as the adherence is seen more on the rough surface topographies. The samples were inoculated with *Candida albicans* at 37°C for 24 hours and incubated for 48 hours followed by examination of the yeast cells using light microscopy. The *Candidal* cell count on different specimens was compared before and after giving probiotics.

Keywords: Acralyn-H, *Candida albicans*, *Candidal* adherence, Dental Products of India, Heat cure denture base resin, Probiotic, Pyrax, Yakult.

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INTRODUCTION

In the present-day scenario, the world is heading toward modern-day epidemics. The number of deaths reported due to cancer is not just limited to developing countries, but has even reached high prevalence among developed countries. Among various cancers, head and neck (HN) cancer in India is emerging as a major public health problem. Head and neck cancer is described as the cancer of the tonsil, pharynx, nasal cavity, salivary gland, hypopharynx, and larynx. Oral cancer (OC) refers to the cancer of the lip, tongue, gingivae, floor of the mouth, palate (hard and soft), maxilla, and cheek. The prevalence of HN cancer with respect to total body malignancy (TBM) varies from 9.8 to 40%. Its frequency is high in Asia and other less-developed countries, and these countries account for nearly 0.7% million new HN cancer cases every year. In India, HN cancer accounts for 20 to 40% of TBM with oral malignancy (9.4%) observed as the most common site by Indian cancer registries. The OC is the sixth most common cause of death in males and seventh in case of females. The overall prevalence of HN and OC cases reported in Rajasthan in a study conducted in the Department of Oncology, SMS Medical Hospital, Jaipur, Rajasthan, India, is 32.18%.[1] With the increase in life expectancy, geriatric problems became more frequent. Candidosis being a common disease among the elderly, the search for products that could help in its treatment is important. It is acknowledged that oral and general health status declines with age. The global population of denture wearers is increasing, so is the incidence of denture biofilm-related problems, such as denture-associated stomatitis, aspiration pneumonia, and malodor. It has been suggested that consumption of probiotic bacteria may improve oral health.[2-5] However, the effects of probiotics on the oral microbiota of denture wearers have received little attention. In edentulous individuals, acrylic prostheses (dentures) provide a hard, nonshedding surface that facilitates bacterial adhesion and subsequent colonization. An essential prerequisite for successful *Candidal* colonization and infection is the ability of the yeast to adhere to the superficial epithelial cells as well as to the fitting surface of the denture, considering the latter as a reservoir of infection. A number of materials have been tried in the fabrication of denture bases. Among them, metals and acrylics are in vogue. Polymethylmethacrylate has been widely employed in the fabrication of dentures. Adherence of *C. albicans* to various denture base materials has been investigated upon. Studies have shown various factors influencing adherence of *C. albicans* to the denture base materials like:

- Surface free energy
- London Van Der Walls forces
- Hydrophobic nature of the material
- Previous nature of the material
- Water sorption property of the material

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The human oral cavity is known to harbor a multitude of organisms. Among them, *C. albicans* has lately become a cause of great concern to the dental profession. Coexistence of *Candida* species, either as a commensal and/or as a pathogen, has attracted the attention of many investigators. *Candida albicans* has been termed as a notorious opportunistic pathogen amongst the *Candida* species. It is so termed owing to its ability to cause an infection when the host defense is either lowered or rendered inadequate. *Candida* is a genus of yeasts. Many species of the genus are endosymbionts of animal hosts including humans. While usually living as commensals, some *Candida* species have the potential to cause diseases. Clinically, the most significant member of the genus is *C. albicans*, which can cause infections in humans (oral candidosis or thrush).

Among the various denture base materials used, there may be a certain degree of variation in the factors influencing the adherence of *C. albicans*. In contrast to the acrylic denture bases, metallic denture bases have the advantage of presenting impervious hydrophobic surfaces and are also considered superior in many respects. However, literature is rather scanty in reference to the adherence of *C. albicans* to metallic denture base materials, so the following study was undertaken to check whether probiotics alter *Candidal* adherence onto the denture base material.

MATERIALS AND METHODS

An *in vitro* study was conducted in the Department of Prosthodontics, Crown and Bridge and Implantology, Jaipur Dental College & Hospital, Jaipur and Department of Microbiology, SMS Medical College, Jaipur, India, to evaluate the adherence of *C. albicans* to various denture base materials.

Sample number: 10/denture base resin

Sample size: 10 mm × 10 mm (length) and 2 mm (width)

Preparation of Heat Cure Specimens

Test samples were fabricated in the following way using a metal die.

A die was manufactured that had 10 slots, and each slot was of dimension 10 mm×10 mm and 2 mm thick[6] (Fig. 1).

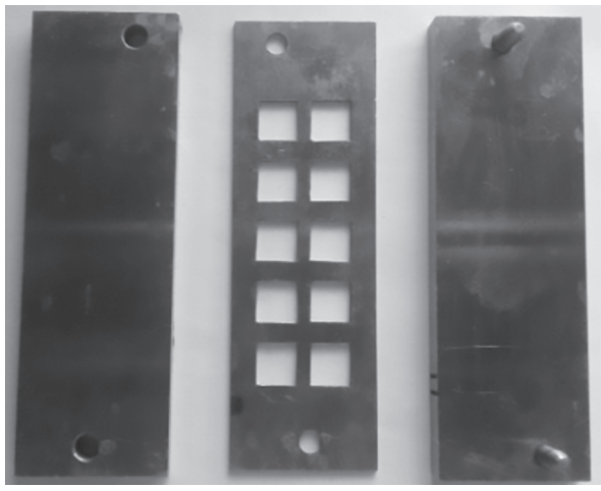


Fig. 1: Metal die

Modeling wax was taken in a glass of metal and melted on top of the flame. The melted wax was poured into the slots of the die and allowed to cool down. After the wax cooled down, removed the excess wax with a wax knife. The process was repeated again and a total of 30 wax squares were made with the help of a die. Flasking was done after the samples were taken out of the die. Heat cure denture base resins of different brands were used at the time of packing, i.e., Acralyn-H, DPI, Pyrax heat cure denture base resin (Fig. 2).

The samples were fabricated by proper mixing of powder and liquid according to the manufacturer's instruction. The flasks were secured for 30 minutes in a dental clamp and polymerized in an acrylizer. A long curing cycle (70° for 9 hours) was employed to decrease the residual monomer content and also obtain good transparency in the material. They were again finished and polished according to the manufacturer's instructions. They were again finished and polished using standard techniques. About 10 samples, each of different material, were placed in three different Petri dishes and labeled accordingly.[7-9]

Saliva Samples and Probiotic Consumption

Totally, 10 patients were included in the study, who have to undergo maxillofacial prosthesis due to surgery, chemotherapy, or radiation and saliva samples were collected

Fig. 2: Different brands of heat cure denture base materials

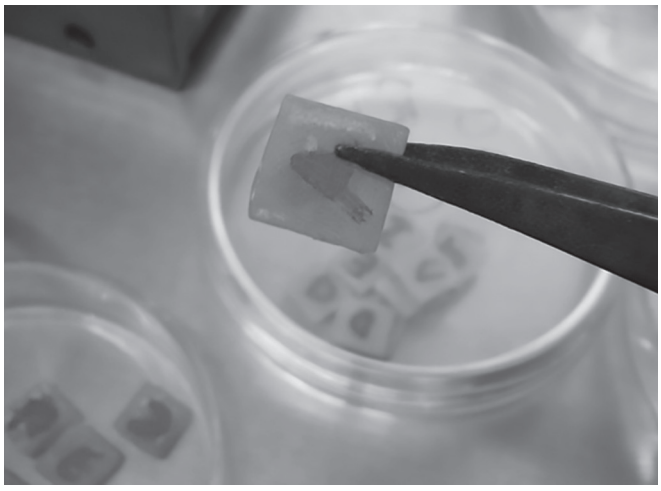


Fig. 3: Labeled individual specimens

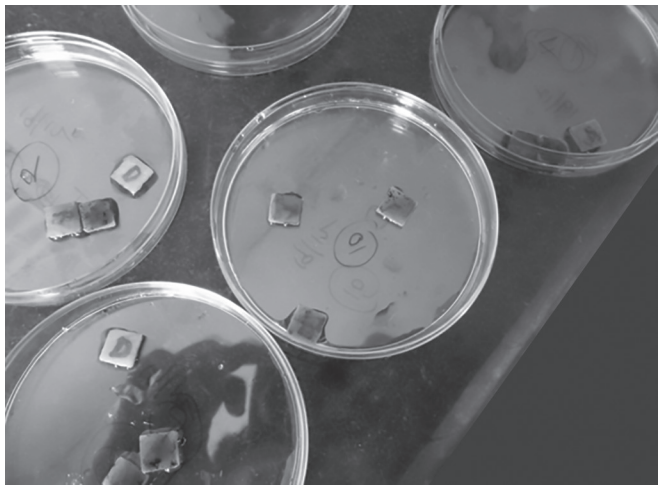


Fig. 4: Stained specimens

from them. Samples of volunteers saliva were collected without stimulation, about 2 hours after oral hygiene, in the morning or in the afternoon. Patients were given probiotic (Yakult probiotic) and were asked to consume it 3 times a week for a period of 1 month.

CULTURE PREPARATION

The culture preparation and the growth of *C. albicans* on the specimens prepared were conducted in the Department of Microbiology, SMS Medical College and Hospital, Jaipur.

Culture Preparation from Patient's Saliva Sample

Patient's saliva sample is picked up from the disposable collector with the help of a nichrome wire loop and introduced into the culture media and incubated for 24 hours. This procedure is carried out inside the ultraviolet chamber. Candidal colonies are formed on the media after 24 hours. Sample is picked up from the colony with the help of the nichrome wire loop and introduced in Sabouraud's broth and placed inside the incubator for 48 hours.

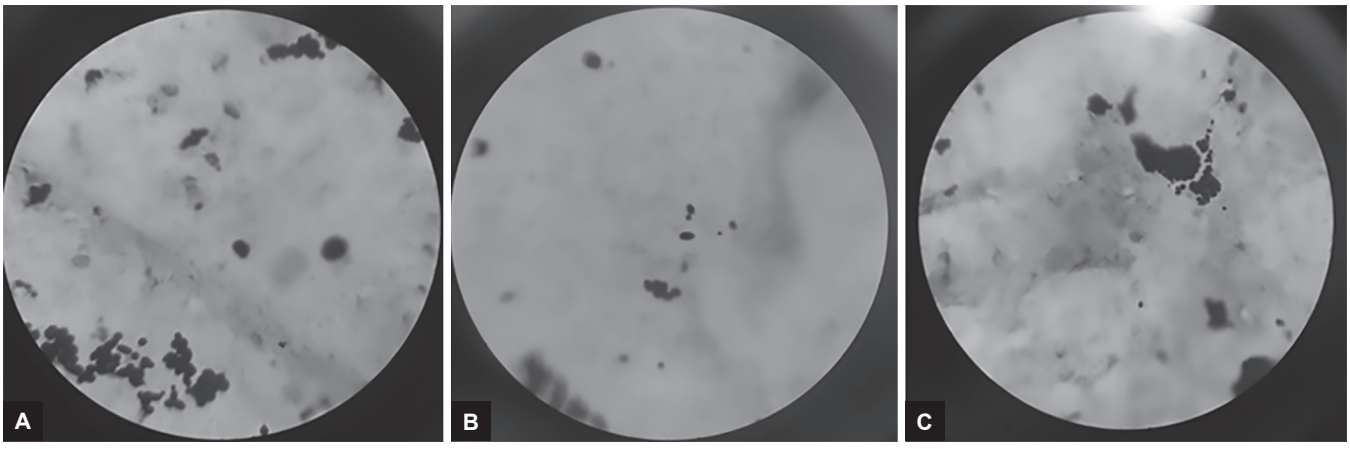
The 48 hours broth culture with *C. albicans* (100 mL) was dispensed in 10 sterile Petri dishes.

To each Petri dish, specimens labeled A, B, C, D, etc. (Fig. 3) respectively, were added, e.g., specimens labeled A of different materials (DPI, Acralyn-H, Pyrax) were added in one Petri dish and specimens labeled B of different materials were added in another Petri dish. Totally, 30 prepared specimens were distributed accordingly in alphabetical order in each Petri dish; all the samples labeled A of different materials were placed in one Petri dish and all the samples labeled B of different materials were placed in another Petri dish, and so on. These were then incubated at 37°C for 48 hours. After completion of the incubation period, the specimens were removed using sterile forceps to avoid any contamination. They were then washed in sterile phosphate-buffered saline (10 Mn phosphate buffer, 2.7 Mn potassium chloride, 137 Mn sodium chloride, pH 7.4). The washing was done gently and for several times to remove loose adherent cells. The specimens were then fixed using sterile methyl alcohol for 1 minute after which the alcohol was drained. The specimens were stained using Gram's staining technique (Fig. 4).

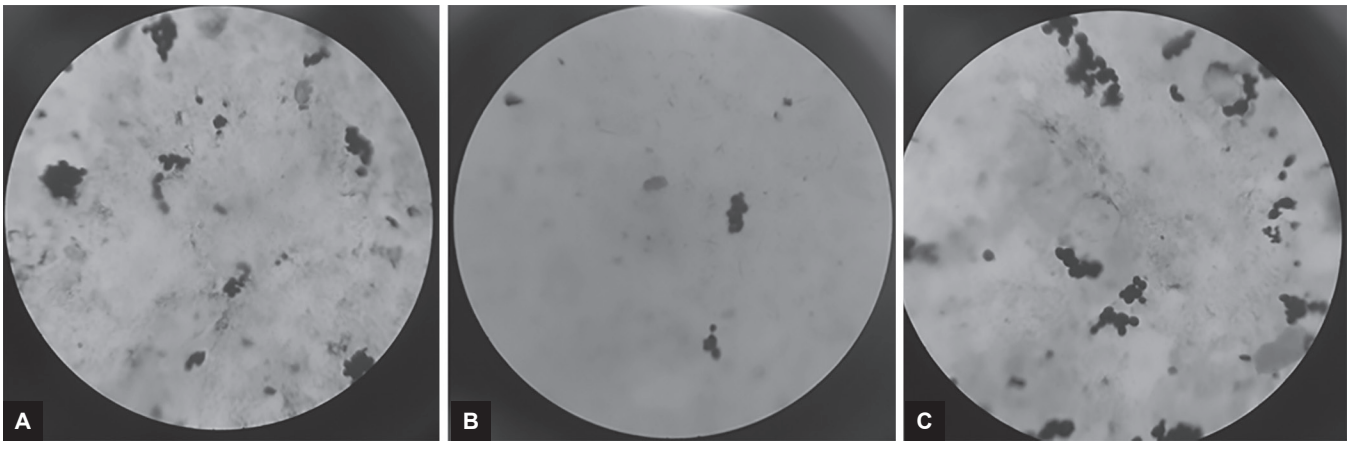
Specimens were washed in water and the stained smear was allowed to dry in air. A drop of cedar-wood oil was placed over the specimen that was placed on a glass slide and observed under oil immersion lens (1000×). The same procedure is repeated as mentioned above and the cells were counted microscopically on the specimen and the *Candidal* cells were compared on different heat cure denture base materials before and after giving probiotic (Figs 5 and 6), and the data are used for statistical analysis. Microscopically, yeast cells are dark purple and show characteristic budding. A total of five random fields were viewed under the light microscope for each of the 10 samples and the fields that showed 0 cell were not included in statistical analysis. After 5 random fields, the number of cells was counted, tabulated as 1 for 1, 2 for 2, 3 for 3, and so on, and used for statistical analysis. After a period of 30 days, another saliva sample was collected from the same patients.

RESULTS

We had prepared 10 samples and three different heat cure denture base materials, i.e., DPI, Pyrax, and Acralyn-H and compared the adherence of *Candida* (*Candidal* colony cell count) onto them, before and after giving probiotic (Table 1). Average *Candidal* cell counts on different heat cure denture base materials before giving probiotic were compared by applying the analysis of variance (ANOVA)



Figs 5A to C: *Candidal* cell count before giving probiotic as seen under a light microscope (patient no. 1)



Figs 6A to C: *Candidal* cell count after giving probiotic as seen under a light microscope (patient no. 1)

Table 1: *Candidal* cell count before and after giving probiotic in maxillofacial patients

	Candidal cell count before giving probiotics			*Candidal cell count after giving probiotics*		
Patient no.	*Pyrax*	*DPI*	*Acralyn-H*	*Pyrax*	*DPI*	*Acralyn-H*
1	60	136	25	33	70	11
2	21	65	11	13	40	7
3	26	68	19	14	35	9
4	22	34	11	12	18	7
5	28	49	10	18	30	5
6	15	22	8	9	16	5
7	19	30	9	11	21	6
8	21	31	13	12	17	6
9	19	26	8	14	22	5
10	16	24	10	13	17	7

Table 2: Average *Candidal* cell count on different heat cure denture base materials before giving probiotics

	n	*Mean*	*Standard deviation*	*p-value*
PYRAX_Pre_Probio	10	24.70	13.030	0.0033
DPI_Pre_Probio	10	48.50	34.923	
ACRALYN_H_Pre_Probio	10	12.40	5.461	
Total	30	28.53	25.937	

Table 3: Average *Candidal* cell counts of different pairs of heat cure denture base materials before giving probiotics

Post hoc tests			
Multiple comparisons			
*Candidal*_Cell_Count			
Tukey HSD			
(I) Group	*(J) Group*	*Mean difference (I–J)*	*p-value*
PYRAX_Pre_Probio	DPI_Pre_Probio	–23.800	0.054
	ACRALYN_H_Pre_Probio	12.300	0.427
DPI_Pre_Probio	PYRAX_Pre_Probio	23.800	0.054
	ACRALYN_H_Pre_Probio	36.100*	0.0026
ACRALYN_H_Pre_Probio	PYRAX_Pre_Probio	–12.300	0.427
	DPI_Pre_Probio	–36.100*	0.0026

*The mean difference is significant at the 0.05 level

test (Table 2) and p = 0.0033, i.e., the difference among average *Candidal* cell counts on different heat cure denture base materials before giving probiotic is significant. To compare the average *Candidal* cell counts of the different pairs of heat cure denture base materials before giving probiotic, we applied the *post hoc* test (Table 3). On applying the *post hoc* test, we found out that average *Candidal* cell count on DPI and Acralyn-H was found significant

Table 4: Average *Candidal* cell counts on different heat cure denture base materials after giving probiotics

	n	*Mean*	*Standard deviation*	*p-value*
PYRAX_Post_Probio	10	14.90	6.773	0.0003
DPI_Post_Probio	10	28.60	16.761	
ACRALYN-H_Post_Probio	10	6.80	1.932	
Total	30	16.77	13.650	

Table 6: Comparison of average *Candidal* cell count of different pairs of heat cure denture base materials both before and after giving probiotics

Paired sample statistics		*Mean*	*n*	*Standard deviation*	*p-value*
Pair 1	PYRAX_Pre_Probio	24.70	10	13.030	0.001
	PYRAX_Post_Probio	14.90	10	6.773	
Pair 2	DPI_Pre_Probio	48.50	10	34.923	0.008
	DPI_Post_Probio	28.60	10	16.761	
Pair 3	ACRALYN_H_Pre_Probio	12.40	10	5.461	0.001
	ACRALYN-H_Post_Probio	6.80	10	1.932	

(p-value=0.0026). We applied the ANOVA test for comparing the average *Candidal* cell counts after giving the probiotic for different heat cure denture base materials (Table 4). The difference in average *Candidal* cell counts on different heat cure denture base material is significant (p-value=0.0003). For comparing the average *Candidal* cell counts among different heat cure denture base materials after giving probiotic, we applied the *post hoc* test. We found out that the average *Candidal* cell count on DPI and Pyrax was significant (p = 0.0186). Similarly, average *Candidal* cell count on DPI and Acralyn-H was also found to be highly significant (p = 0.0002) (Table 5). The adherence of *C. albicans* was seen to be significantly low in Acralyn-H heat cure denture base material, followed by Pyrax heat cure denture base resin, which was followed by DPI heat cure denture base resin (Table 6). There was a significant decrease in *Candidal* cell counts after giving probiotics on the different heat cure denture base materials.

DISCUSSION

This study was done to evaluate the effect of probiotics on *Candidal* adherence on different denture base resins in maxillofacial patients. Three different types of heat cure denture base resins were used (namely, Acralyn-H, DPI, and Pyrax). The saliva samples were collected from the maxillofacial cancer patients, who have undergone chemotherapy, surgery, etc., and the patients were given probiotics and asked to consume it 3 times a week for a period of 1 month. *Candidal* colony cell count was done on different heat cure denture base materials both before and after giving probiotics to the maxillofacial patients

Table 5: Average *Candidal* cell count of different pairs of heat cure denture base materials after giving probiotics

Post hoc tests			
Multiple comparison			
*Candidal*_Cell_Count			
Tukey HSD			
(I) Group	*(J) Group*	*Mean difference (I–J)*	*p-value*
PYRAX_Post_Probio	DPI_Post_Probio	–13.700*	0.0186
	ACRALYN-H_Post_Probio	8.100	0.2142
DPI_Post_Probio	PYRAX_Post_Probio	13.700*	0.0186
	ACRALYN-H_Post_Probio	21.800*	0.0002
ACRALYN-H_Post_Probio	PYRAX_Post_Probio	–8.100	0.2142
	DPI_Post_Probio	–21.800*	0.0002

*The mean difference is significant at the 0.05 level

and the results obtained were compared and used for statistical analysis. In a study done by Kalla et al[10] on the surface adherence of *C. albicans* to different PMMA denture base resins, the values of the test results showed that the statistical analysis was highly significant. Hence, it was refuted that the null hypothesis was at 0.1% level of significance (p<0.001), and it was concluded that the materials used in groups I, II, III, IV, and V vary significantly. The adherence of *C. albicans* was seen to be significantly low in Lucitone 199 heat cure denture base material, followed by DPI heat cure denture base resin, which was followed by Trevalon Clear heat cure denture base resin. Significantly high adherence was noted in DPI self-cure PMMA denture base resin and light cure denture base material, but the self-cure showed more. However, in our study, we have compared the *Candidal* adherence on different heat cure denture base resins, and the statistical analysis was found to be highly significant. The adherence of *C. albicans* was the least on Acralyn-H, which was followed by Pyrax heat cure denture base material, and the highest *Candidal* adherence was reported on DPI heat cure denture base material. Statistical analysis showed that the difference in *Candidal* adherence between DPI and Acralyn-H before giving the probiotic was highly significant (p-value=0.0026). In both the studies, there is a significant difference in *Candidal* adherence on different denture base materials due to the difference in properties of different materials. *Candidal* adherence is different on different heat cure denture base materials as features, such as water sorption and permissive surface may alter the degree of adherence of *C. albicans* to acrylics. It has been reported that other factors, such as surface free energy, hydrostatic forces, hydrophilic or hydrophobic nature of the material, previous nature of the material, and water sorption affect the nature of adherence of the yeast to the denture base materials. In

a study done by Hatakka et al,[11] they found out the effect of probiotics on the prevalence of *Candida* in the elderly. During this 16-week, randomized, double-blind, placebo-controlled study, 276 elderly people consumed daily 50 gm of either probiotic (n=136) or control cheese (n = 140). The primary outcome measure was the prevalence of a high salivary yeast count [$>10^4$ colony-forming units (CFU)/mL] analyzed by the Dentocult® method. The prevalence decreased in the probiotic group from 30 to 21% (32% reduction), and increased in the control group from 28 to 34%. Probiotic intervention reduced the risk of high yeast counts by 75% [odds ratio (OR) = 0.25, 95% confidence interval (CI) 0.10–0.65, p = 0.004] and the risk of hyposalivation by 56% (OR = 0.44, 95% CI 0.19-1.01, p = 0.05). Measurement of hyposalivation was not considered in our study. Thus, probiotic bacteria can be effective in controlling oral *Candida* and hyposalivation in the elderly. In this study, 276 elderly people were included in the study and 136 elderly people were administered probiotics, whereas, in our study, only 10 saliva samples from maxillofacial patients were included and *Candidal* adherence was evaluated on different heat cure resins before and after giving probiotics, There was a significant decrease in *Candidal* cell count after giving the probiotic (Before, pre-probiotic p=0.0033, after, post-probiotic, p = 0.0003). The reduction in *Candidal* colony cell count is seen in both the studies as probiotics have an inhibitory effect on *Candidal* growth. In a study done by Mendonça et al[2] on the effects of probiotic bacteria on *Candida* presence and immunoglobulin A (IgA) anti-*Candida* in the oral cavity of the elderly. Here, the effect of probiotics on the growth of *C. albicans* in healthy elderly patients was shown, whereas, in our study, we have evaluated the effect of probiotics in immunocompromised patients, who had undergone cancer, surgery, chemotherapy, radiation, etc. Imbalance in the resident microbiota may promote the growth of opportunistic microorganisms, such as yeasts of *Candida* genus and the development of diseases, especially in aged people. Their study evaluated whether the consumption of the probiotic Yakult LB® (*Lactobacillus casei* and *Bifidobacterium breve*) was able to influence the specific immunological response against *Candida* and the presence of these yeasts in the oral cavity. Anti-*Candida* IgA analysis was conducted using the enzyme-linked immunosorbent assay technique, whereas, in our study, we did not measure the IgA levels. The ANOVA and Student's t-test were used for normally distributed data and the Wilcoxon test was used for data with non-normal distribution ($\alpha = 0.05$). Whereas, in our study, we applied the ANOVA test and the *post hoc* test for the statistical evaluation of *Candidal* colony cell counts on different heat cure denture base materials before and after giving probiotic in maxillofacial patients. The results showed a statistically significant reduction ($p < 0.05$) in *Candida* prevalence (from 92.9 to 85.7%), in CFU/mL counts of *Candida* and in the number of non-*albicans* species after consumption of the probiotic. Immunological analysis demonstrated a significant increase ($p < 0.05$) in anti-*Candida* IgA levels. In conclusion, probiotic bacteria reduced *Candida* numbers in the oral cavity of the elderly and increased specific secretory immune response against these yeasts, suggesting its possible use in controlling oral candidosis. In our study, there was a statistically significant decrease in average *Candidal* cell count ($p<0.05$) after giving probiotics. We have found matches between findings of our study and the findings of the study done by Mendonça et al. The difference was that we had counted the number of *Candidal* cells on the different heat cure denture base materials before and after giving probiotics in the maxillofacial patients, and only the *C. albicans* species was included in our study. In both the studies, there is a reduction in *Candidal* cell count after giving probiotics due to the inhibitory effect of probiotics on the *Candidal* growth.

CLINICAL SIGNIFICANCE

This article is an original study done on the effect of probiotics on *Candidal* adherence on different heat cure denture base resins. Maxillofacial prosthesis is widely used in patients who are immunocompromised due to chemotherapy, surgery, radiation, etc. Patients have maxillofacial defects for which they need replacement. Maxillofacial prosthesis like obturators, etc., provide such replacement of the defect, and in such patients, there is high prevalence of *Candidal* growth on the prosthesis. Probiotics inhibit the growth of *Candida*, and, as a result, there is significantly decreased *Candidal* growth on the prosthesis. Thus, the major mechanisms of anti-*Candida* activity are due to probiotic nutritional competition, blocking the receptors for adhesins of *Candida sp* epithelial cells, adhesion to epithelial cells causing competition for adhesion site, increased intestinal peristalsis, increased rate of renewal of intestinal epithelial cells, changes in pH, bacteriocin production (cationic, hydrophobic, and heterogeneous with bactericidal and bacteriostatic effects), coaggregation molecules, lactic acid, acetic acid, hydrogen peroxide, and biosurfactants that resulted in the inhibition of growth of pathogen.[4]

SUMMARY AND CONCLUSION

The study was undertaken with the objective of evaluating the effect of probiotics on *Candidal* adherence on different heat cure denture base materials in maxillofacial patients. Ten samples for each type of the heat cure

PMMA were fabricated respectively. Totally, 60 samples were considered, 30 samples before giving probiotic and 30 samples after giving probiotic. The adherence of the yeast on the tissue surface of the specimens was given the prime consideration as the adherence is seen more on the rough surface topography. As such, the tissue surface remained untouched. The samples were inoculated with *C. albicans* at 37°C for 24 hours and incubated for 48 hours followed by examination of the yeast cells using light microscopy. The *Candidal* cell count on different specimens was compared before and after giving probiotic.

The conclusions of our study, therefore, are as follows:

- Acralyn-H heat cure PMMA denture base resins showed the least adherence of *Candidal* cells compared with other heat cure denture base materials.
- After Acralyn-H, Pyrax heat cure denture base resin showed less adherence followed by DPI heat cure denture base resin.
- *Candidal* cell count was significantly reduced on different heat cure denture base materials after giving probiotics to the maxillofacial patients.

Probiotics play a major role in reducing *Candidal* cell count inside the oral cavity in immunocompromised patients.

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