ORIGINAL ARTICLE

COMPARATIVE STUDY OF EFFECTIVENESS OF DISINFECTANTS ON CANDIDA ALBICANS

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HOW TO CITE THIS ARTICLE:

B. Naga Jyothi, P. Venkateswarlu, B. Shanti Reddy, S. Umadevi. "Comparative Study of Effectiveness of Disinfectants on Candida Albicans". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 04, January 12; Page: 636-643, DOI: 10.14260/jemds/2015/93

ABSTRACT: A considerable proportion of the population have detectable numbers of yeast in the mouth, yet very few of those people suffer from oral Candida infection. Placement of removable dentures in the oral cavity produces profound changes of the oral environment that may have an adverse effect on the integrity of the oral tissues, which might further change the oral microbial flora. Improper oral hygiene and negligence on denture care lead to candidiasis. The denture cleansers currently available in the market are costly and are not readily available. The current study attempted at evaluating the effectiveness of house hold denture cleansers on Candida albicans.

KEYWORDS: Candida albicans, Dentures, Denture cleansers, Culture, Colony counting.

INTRODUCTION: A considerable proportion of the population have detectable numbers of yeast in the mouth, yet very few of those people suffer from oral Candida infection. Placement of removable dentures in the oral cavity produces profound changes of the oral environment that may have an adverse effect on the integrity of the oral tissues, which might further change the oral microbial flora. Improper oral hygiene and negligence on denture care lead to candidiasis¹. The denture cleansers currently available in the market are costly and are not readily available. The current study attempted at evaluating the effectiveness of house hold denture cleansers on Candida albicans².

MATERIAL AND METHODS: The present study was conducted in the Dept of Microbiology, Kurnool Medical College, Kurnool during 2013-2014.

An in vitro study was conducted to evaluate and compare the effectiveness of various household denture cleanser solutions against candida albicans.

STEPS FOLLOWED IN THE STUDY:

- 1. FABRICATION OF MASTER DIE FOR PREPARATION OF STUDY MOULD:** For the study a precise, metal mould was custom fabricated with measurement of 10x10x2 mm,, and was used to make test samples.
- 2. PREPARATION OF DENTAL PLASTER MOULD SPACE:** Wax patterns were obtained by pouring wax into the die space present within the metal mould. The wax patterns obtained were checked for voids and inaccuracies. The wax patterns were then invested in a dental flask, using dental plaster following the manufacturer's instructions for water-powder ratio, mixing time and setting time. Mechanical vibrator was used to prevent air trapping during investing. Once the plaster was completely set, dewaxing was done for 10 min. The mould space thus obtained was used for the preparation of the test samples.
- 3. PREPERATION OF TEST SAMPLES:** The denture base resins used were in ratio of 1:3 monomer and polymer for all the groups. Separating medium was applied on to the dental

ORIGINAL ARTICLE

plaster mould with the help of a camel hair brush and dried. The material was mixed following the manufacturers' instructions and packed at dough stage. The specimens were bench cured for 1 hr and polymerized by short curing cycle according to manufactures instructions. After processing, the specimens were bench cooled for 1 hr and then finished with various grits of sand paper, followed by polishing with pumice. Thickness, length and width of each specimen were verified. The specimens were stored in distilled water for 24 hrs.

GROUPING OF STUDY SPECIMENS:

SL. NO	GROUP	COMPONENT	NO. OF SAMPLES
1	A Denture Cleanser-1 (hydrogen peroxide)	Heat polymerized acrylic resin specimens immersed in denture cleanser solution hydrogenperoxide for 8hrs.	10
2	B Denture Cleanser-2 (alcohol, citricacid, bakingsoda)	Heat polymerized acrylic resin specimens immersed in denture cleanser solution (alcohol, citricacid, bakingsoda) for 8hrs	10
3	C Denture Cleanser-3 (household bleach)	Heat polymerized acrylic resin specimens immersed in denture cleanser solution of household bleach 30 min	10
4	Denture Cleanser-4 (10%vinegar)	Heat polymerized acrylic resin specimens immersed in distilled water for 24 hours.	10
5	Control Group (distilled water)	Heat polymerized acrylic resin specimens immersed in distilled water for 24 hours.	10

STERILIZATION OF ACRYLIC RESIN SPECIMEN: The acrylic resin specimens will be sterilized in ultraviolet light chamber for 5 minutes. As ultraviolet light does not penetrate opaque materials, the specimens will be overturned using sterile forceps and the other side will be sterilized for 5 minutes. The acrylic specimens will be then placed in sterilization pouches.

BROTH PREPARATION: Two to three colonies of *Candida albicans* grown on blood agar will be taken and incubated onto sabourards dextrose broth. This will be incubated aerobically at 37°C for 24 hours and should be matched to mcfarlands stand and unit 1.

Figure 1

ORIGINAL ARTICLE

PREPARATION OF DENTURE CLEANERS:

- 20ml of 3% H₂O₂
- 10ml Alcohol+1teaspoon fullsoda+10ml citric acid.
- 1 teaspoon full washing soda+20ml distilled water.
- 20ml 10%vinegar.
- 20ml normal tap water.(for control group).

EXPOSURE OF SPECIMENS TO CANDIDA ALBICANS: The sterile acrylic resin specimens of various groups will be immersed, with labelled surface facing down, in Petri dishes containing 20ml of Sabouraud dextrose broth containing Candida albicans. These will be incubated for 16 hours at 37°C in incubator. This will simulate the duration of denture worn by the patient during daytime. Then the inoculated specimens will be washed under running tap water to simulate the patient's routine denture cleaning procedure.

Figure 2

CANDIDA REMOVAL TEST: The specimens will be immersed in Petri dishes containing denture cleaning agents. They will be stored for 8 hours at room temperature. This mimics the overnight soaking of dentures in cleaners according to the manufacturer's instructions. After this the specimens will be washed under running tap water, will be fixed with methanol, stained with crystal violet, dried and will be examined under microscope.

COUNTING OF CANDIDA CELLS: Candida cells adherent to acrylic resin specimens will be counted under the microscope(x40 magnification). The number of cells adherent on the test samples will be compared with that adherent to control.

Figure 3

ORIGINAL ARTICLE

STATISTICAL ANALYSIS: Data collected by experiments were computerized and analyzed using the Statistical Package for Social Sciences (SPSS) version 15.0. Results were expressed in frequencies. Nonparametric tests namely ANOVA test and tukey test were used for testing the statistical significance. For all tests a p-value of 0.05 or less was considered for statistical significance.

RESULTS:

Figure 4

Table: Comparison of five groups (H₂O₂, Alcohol, House Hold Bleach, 10% Vinegar and Water) with respect to CFU counts in difference from before and after immersion in denture cleanser by one way ANOVA.

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Between groups	6172.7200	4	1543.18	26.9065	0.00001*
Within groups	2580.9000	45	57.35		
Total	8753.6200	49			

*p<0.05

Figure 5

DISCUSSION: Maintaining oral hygiene is essential even when some or all teeth have been replaced with removable dentures. A properly maintained prosthesis contributes to good oral health. With patient education and preventive dentistry, a dentist must be able to recommend a denture cleanser that is effective, shows less deleterious effects on denture base materials and compatible with oral tissues.³

The process by which dentures accumulate plaque, stain and calculus is apparently similar to that process which takes place on natural teeth. Tonzetich studied the causes of halitosis extensively and concluded that a program of good oral hygiene can effectively control odor in most instances of oral origin. Theliade suggested that the presence of denture plaque constitutes the principal cause leading to inflammation of the palatal mucosa.

According to Budtz-Jorgensen this inflammation in edentulous patients can best be prevented by meticulous oral and denture hygiene.⁴

An ideal denture cleanser should have bactericidal and fungicidal properties, be simple to use, effectively remove organic and inorganic matter from denture surfaces, and be compatible with all denture materials.^{5,6} Denture cleansers are widely used to prevent colonization by *Candida albicans* and related *Candida* species, and to prevent denture plaque formation.^{7,8}

Chemical cleansing systems can be divided into 5 different groups, depending on their chemical constituents and mechanism of action. These include alkaline peroxides, alkaline hypochlorites, acids, disinfectants, and enzymes. Acrylic resin specimens of the size 10x10x2mm were fabricated using customized metal mould. A total of 50 standardized specimens were fabricated and stored in distilled water for 24hrs. After 24 hrs, the specimens were appropriately labelled and immersed in groups of 10 to prepared denture cleanser solutions for treatment. Solutions were prepared and immersed according to manufactures instructions. The removal of adherent cells from the denture base surface is an important part of denture cleansing.⁹ The *Candida* removal test is a

ORIGINAL ARTICLE

quantitative measurement of the efficacy of denture cleanser.¹⁰ As such, in this study Candida removal test was performed to check the efficacy of denture cleaning agents. The present study evaluated and compared effectiveness of different denture cleansers in removing Candida from the surface of acrylic resin specimens.

After preparation of specimens Two to three colonies of *Candida albicans* grown on blood agar will be taken and incubated onto sabourauds dextrose broth. This will be incubated aerobically at 37°C for 24 hours. Candidal growth is seen in sabourauds dextrose broth and is matched to mcfarlands standard 1 which is equal to 1×10^6 candidal cells per millilitre. Exposure of specimens to *Candida albicans* present in broth the sterile acrylic resin specimens of various groups by immersing in to broth with labelled surface facing down, in Petridishes containing 20ml of Sabouraud dextrose broth containing *Candida albicans*. These will be incubated for 16 hours at 37°C in incubator. This will simulate the duration of denture worn by the patient during daytime. Then the inoculated specimens will be washed under running tap water to simulate the patient's routine denture cleaning procedure. Then candidal colony count of each specimen is calculated and noted as initial colony count of 50 specimens. After that Candida removal test is done by immersing the specimens in Petridishes containing denture cleaning agents. They will be stored for 8 hours at room temperature. This mimics the overnight soaking of dentures in cleaners according to the manufacturer's instructions. After this the specimens will be washed under running tap water, will be fixed with methanol, stained with crystal violet, dried and will be examined under microscope. Candida cells adherent to acrylic resin specimens will be counted under the microscope (x40 magnification). The number of cells adherent on the test samples will be compared with that adherent to control and the initial count obtained while immersion into the sabourauds dextrose broth. The findings for all the groups were tabulated and statistically analyzed using POST HOC TUKEY TEST, ONE WAY ANOVA TEST, PAIRED "T" TEST was done. The 'P' value of ≤ 0.05 was considered significant. As seen from table 1 and graph 1, group the control (n=10) consisting of denture base acrylic specimens immersed in distilled water (10×10×2 mm), The mean colony count of candida on denture base acrylic specimens after immersion in Group A (hydrogen peroxide denture cleansers) ranged from a maximum of 70.50 before immersion, to a minimum of 50 after immersion into denture cleanser,. The mean colony count of candida on denture base acrylic specimens after immersion in Group b (alcohol, bakingsoda, citricacid combination denture cleansers) ranged from a maximum of 83.20 before immersion, to a minimum of 47 after immersion into denture cleanser. The mean colony count of candida on denture base acrylic specimens after immersion in Group c (household bleach denture cleansers) ranged from a maximum of 79.50 before immersion, to a minimum of 40.30 after immersion into denture cleanser. The mean colony count of candida on denture base acrylic specimens after immersion in Group d (vinegar denture cleansers) ranged from a maximum of 83.60 before immersion, to a minimum of 48.40 after immersion into denture cleanser. The mean colony count of candida on denture base acrylic specimens after immersion in Group e (water *control) ranged from a maximum of 90.10 before immersion, to a minimum of 79.90 after immersion into denture cleanser. As seen from Table:2 Comparison of five groups (H_2O_2 , Alcohol, House Hold Bleach, 10% Vinegar and Water) with respect to CFU counts in before immersion in denture cleanser by one way ANOVA between groups and within groups, the "p" value is 0.01 which is less than 0.05 which is statistically significant. As seen from the Table:3 Pair wise comparison of five groups (H_2O_2 , Alcohol, House Hold Bleach, 10% Vinegar and Water) with respect to CFU counts before immersion in denture

ORIGINAL ARTICLE

cleanser by Tukeys multiple post hoc procedures the “p” value is less than 0.05 when compared pair wise between groups, so stastically significant.

CONCLUSION:

Within the limitations of the study, the following conclusions were drawn:

The colony count decreases after immersion in to household denture cleanser solutions like baking soda, Sodium hypochlorite, alcohol, vinegar, household bleach, and were clinically significant.

House hold bleach showed more reduction in colony count of candida albicans followed by alcohol +baking soda+lemon juice combination,vinegar,H₂O₂ and water respectively.

ACKNOWLEDGEMENT: Authors are thankful to Department of Microbiology, KMC, Kurnool.

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ORIGINAL ARTICLE

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Date of Submission: 05/01/2015.
Date of Peer Review: 06/01/2015.
Date of Acceptance: 07/01/2015.
Date of Publishing: 09/01/2015.