

# VINEGAR AS AN ANTIMICROBIAL AGENT FOR CONTROL OF *Candida* spp. IN COMPLETE DENTURE WEARERS

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## ABSTRACT

The use of denture is known to increase the carriage of *Candida* in healthy patients, and the proliferation of *Candida albicans* strains can be associated with denture-induced stomatitis. The aim of this study was to evaluate the use of vinegar as an antimicrobial agent for control of *Candida* spp. in complete upper denture wearers. Fifty-five patients were submitted to a detailed clinical interview and oral clinical examination, and were instructed to keep their dentures immersed in a 10% vinegar solution (pH less than 3) overnight for 45 days. Before and after the experimental period, saliva samples were collected for detection of *Candida*, counting of cfu/mL and identification of species by phenotypical tests (germ tube formation, chlamidoconidia production, and carbohydrate fermentation and assimilation). The results were analyzed using Spearman's correlation and Student's t-test ( $p \leq 0.05$ ). *Candida* yeasts were present in 87.3% of saliva samples before the treatment. A significant reduction was verified in CFU/mL counts of *Candida* after treatment. A positive correlation between *Candida* and denture stomatitis was verified, since the decrease of cfu/mL counts was correlated with a reduction in cases of denture stomatitis. Although it was not able to eliminate *C. albicans*, the immersion of the complete denture in 10% vinegar solution, during the night, reduced the amounts (cfu/mL) of *Candida* spp. in the saliva and the presence of denture stomatitis in the studied patients.

**Key words:** *Candida*. *Candida albicans*. Denture. vinegar.

## INTRODUCTION

Although dentistry has developed new materials and techniques used in the rehabilitation of completely edentulous patients, mucosa-supported dentures still act as possible agents of tissue damages. The continuous use of dentures and the lack of regular monitoring provide the development of injuries<sup>11,19</sup>, stomatitis being the most frequently observed.

Denture-induced stomatitis is an inflammatory reaction of the denture-bearing mucosa that affects approximately 65% of complete upper denture wearers<sup>28</sup> and is characterized by different degrees of erythema<sup>1,6,8,12</sup>. It has a multifactorial etiology, and *C. albicans* is reported as the primary etiologic agent<sup>1,21</sup>.

Poor hygiene of dentures is also associated with the disease<sup>4</sup>, demonstrating the importance of denture cleansing by mechanical and/or chemical methods. The most commonly used mechanical methods are brushing with

dentifrice or neutral soap and use of ultrasonic devices. The chemical methods involve denture immersion in chemical products, such as sodium perborate and sodium hypochlorite, hydrochloride, phosphoric and benzoic acids, chlorhexidine digluconate, and enzymes, like proteases and mutanases<sup>2,3</sup>.

Despite the large number of currently available commercial denture-cleansing products, less than 60% of the wearers make use of anyone<sup>4</sup>, possibly because of the low purchasing power of great part of the Brazilian population.

The vinegar is a sour and astringent liquid consisting mainly of acetic acid, resulting from the fermentation of an alcoholic beverage, mainly white and red wines. This product is cheap, easily found in the market, and seems to have antimicrobial potential<sup>18,26,27,30</sup>. Estrela, et al.<sup>9</sup> (2004) evaluated the use of vinegar as an irrigating solution and intracanal medicament in microbial elimination from dog's teeth with apical periodontitis. Microbe absence was observed in 40% of samples treated with vinegar.

Basson, et al.<sup>2</sup> (1992) showed the effectiveness of undiluted vinegar solutions in killing adherent microorganisms when used as disinfection agent for denture cleansing. *In vitro* experiments have already shown that low fungicidal doses of acetic acid induce programmed cell death in *C. albican*<sup>22</sup>.

The aim of this study was to verify the effectiveness of a 10% vinegar solution in the control of *Candida* spp. in oral cavity of complete upper denture wearers, evaluating the presence of stomatitis and *Candida* yeast counts in saliva.

## MATERIAL AND METHODS

After approval by the local Research Ethics Committee, 55 patients of both genders aged 32 to 81 years old, wearers of complete upper denture for more than 6 months, were invited to participate in this study. The patients informed the time of denture use. The exclusion criteria were: absence of yeasts in oral cavity, presence of diabetes mellitus, arterial hypertension or xerostomy, and use of antifungal, antibiotic and antidepressant drugs in the 6 months preceding the study. All the participants were informed about the study objectives and methodology and signed an informed consent form.

After detailed clinical interview, two previously calibrated examiners investigated the occurrence of stomatitis and other injuries on oral mucosa. Denture-induced stomatitis, if present, was assessed according to Newton's classification, which is based on the clinical appearance of the inflamed mucosa under complete dentures: Type I: petechiae dispersed throughout all or any part of palatal mucosa in contact with the denture; Type II: diffuse erythema confined to the denture bearing area; and Type III: papillary hyperplasia with diffuse erythema confined to the denture bearing area<sup>20</sup>. The examiners also evaluated the hygiene of the dentures, considering it unsatisfactory when debris, biofilm accumulation or both were observed in any region of dentures.

After that, a sample (2 to 3 mL) of non-stimulated saliva was collected from each patient, with the denture in place, using a disposable sterile collector. The samples were kept in ice and thermal bag during the transportation to the microbiology laboratory, respecting the period of 3 h between sample collection and processing.

Subjects were instructed to remove their denture at night (during approximately 8 h), to brush the denture at least 3 times a day during 1 min, after meals, using an appropriate toothbrush (Bitufe, Itupeva, SP, Brazil) and neutral coconut soap (Dias D'Ávila, Dias D'Ávila, Ba, Brazil) and, after that, to keep the denture immersed in 100 mL of a 10% vinegar solution (pH<3). The brushes, soap and vinegar solution were provided to patients by the authors. The solution was prepared using a red wine vinegar base (Minhoto, Raymundo da Fonte Group, Torres Galvão, Paulista, PE, Brazil) with 4% volatile acidity, which was diluted in sterile distilled water. The pH was measured using a pH meter (Micronal Ind. Ltd., Piracicaba, SP, Brazil), confirming an acidity of less than 3. The vinegar solution

was replaced for a fresh one daily. The experimental period had the duration of 45 days and after this time the patients were reexamined and new saliva sample was collected from each participant.

All saliva samples (0.1 mL) were plated in duplicate in Sabouraud Dextrose agar (Difco Laboratories, Detroit, MI, USA) added with chloramphenicol (0.1 mg/mL), and incubated at 37°C for 48 h. After that, the number of colonies forming units per milliliter of saliva (cfu/mL) was counted. The presence of yeasts in suggestive colonies was confirmed after preparation of Gram-stained smears and observation under optical microscopy. Pure cultures in Sabouraud Dextrose agar were obtained from three different colonies of each morphology, which were identified by phenotypical tests<sup>24</sup>, as follows: germ tube formation in sterile human sera; chlamidoconidia production in Corn Meal Agar (Difco) added with 1% Tween 80 (Interlab Distribuidora de Produtos Científicos S.A., São Paulo, SP, Brazil); sugar fermentation (glucose, galactose, sucrose, maltose and lactose) in phenol red broth (Difco) and sugar assimilation in chemically defined culture medium.

The data of the first collection were submitted to the Kruskal-Wallis test to evaluate if the time of denture use was related with the cfu/mL counts. Student's t-test was applied to verify the effect of the treatment in cfu/mL counts. Significance level was set at 5%. Biostat statistical software (Bioestat, Goiânia, GO, Brazil) was used for both tests. In order to correlate the presence of stomatitis with the cfu/mL counts, before and after the treatment, the data were submitted to Spearman's correlation using the SAS statistical software (Statistical Analysis System; SAS Institute Incorporation, Campus Drive Cary, NC, USA).

## RESULTS

The 55 patients enrolled in this study had a mean age of 57±11.3 years, and were using the complete upper denture for 6 months to 25 years (mean of 7.6 years). Yeasts of *Candida* genus were present in 48 (87.3%) patients, from whom 62 isolates were obtained. Patients who showed no yeasts in the oral cavity were instructed to follow the treatment, but were excluded from the study.

Comparison between the mean of cfu/mL of *Candida* yeasts, before and after the treatment, is shown in Table 1. The obtained t value was positive ( $t=8.91880$ ), indicating a significant reduction in cfu/mL counts of *Candida* after the use of vinegar solution.

A reduction in the incidence of stomatitis was observed after the treatment of the dentures with the vinegar solution (Table 2). The correlation between the number of microorganisms and presence of stomatitis before and after the use of vinegar solution can be observed in Table 3. The results of Spearman's correlation demonstrated a positive correlation between the presence of *Candida* and denture stomatitis after the use of vinegar solution ( $p<0.05$ ), that is, the reduction of cfu/mL counts was correlated with a reduction in the number of patients with denture stomatitis

(34 before and 14 after the use of the vinegar solution). The age of the patients did not show any correlation with the number of *Candida* in saliva. Angular queilitis was present in 19 patients (34.6%) before the treatment and this result did not change after the use of vinegar. Other injuries were not observed in studied patients.

In the second saliva collection, the number of *Candida* strains decreased by 29% (from 62 to 44). Before the treatment, 10 patients showed two species of *Candida* in saliva and 2 other patients showed an association of 3 species (Figure 1). After the treatment, only one patient showed association of 2 species (*C. albicans* and *C. krusei*). *C. guilliermondii*, *C. lusitaniae* and *C. parapsilosis* strains were absent after the use of vinegar solution. The isolated species, before and after

vinegar use, are displayed in Table 4.

The Kruskal-Wallis test did not show significant difference between the mean cfu/mL of the first collection and the time of denture use. The obtained results were: time of denture use between 0.5 and 3 years: mean cfu/mL = 1063.3; between 4 and 8 years: mean cfu/mL = 1039.3; between 9 and 15 years: mean cfu/mL = 1056.6; and between 16 and 25 years: mean cfu/mL = 1073.7 (p=0.9107).

An important decrease in the number of patients with unsatisfactory hygiene after the use of vinegar was observed. Before the treatment, all 48 patients presented poorly cleaned dentures, and this number dropped to 8 after the use of vinegar.

**TABLE 1-** Mean of cfu/mL and standard deviation of *Candida* genus yeasts, before and after the use of vinegar solution (Student's t-test for paired samples)

	Before the use of vinegar solution (n=48)	After the use of vinegar solution (n=48)
Mean ± Standard deviation	1251.02 ± 667.34	451.25 ± 380.19*
Standard deviation of the difference	621.27	-
Mean of the differences	799.77	-
t value	8.9188	-

\*Statistically significant difference ( p ≤ 0.05).

**TABLE 2-** Percentage of patients with or without denture-induced stomatitis, before and after the use of vinegar

Vinegar use (n=48)	With stomatitis			Without stomatitis	
	Newton Classification			Total	Total
	I	II	III		
Before	17(35.4%)	14 (29.16%)	3 (6.3%)	34 (70.8%)	14 (29.2%)
After	19 (39.6%)	3 (6.3%)	1 (2.1%)	23 (47.9%)	25 (52.1%)

**TABLE 3-** p values of correlation between cfu/mL counts in saliva and presence of denture-induced stomatitis, before (BV) and after (AV) the treatment with vinegar (Spearman's correlation)

	cfu/mL BV	cfu/mL AV	Denture stomatitis BV	Denture stomatitis AV
cfu/mL Before Vinegar (BV)	-	0.0078	0.5675	0.3334
cfu/mL After Vinegar (AV)	0.0078	-	0.1798	0.0442
Denture stomatitis Before Vinegar (BV)	0.5675	0.1798	-	<.0001
Denture stomatitis After Vinegar (AV)	0.3334	0.0442	<.0001	-

Statistically significant if p ≤ 0.05.

**TABLE 4-** Species of *Candida* genus isolated from oral cavity of 48 patients, before and after the use of vinegar solution

Species	Before the use of vinegar solution		After the use of vinegar solution	
	Number of strains	%	Number of strains	%
<i>C. albicans</i>	37	59.7	34	77.3
<i>C. glabrata</i>	8	12.9	3	6.8
<i>C. tropicalis</i>	6	9.7	4	9.1
<i>C. krusei</i>	6	9.7	3	6.8
<i>C. guilliermondii</i>	3	4.8	-	-
<i>C. lusitaniae</i>	1	1.6	-	-
<i>C. parapsilosis</i>	1	1.6	-	-
Total	62	100	44	100

Patients (n)	Associations of <i>Candida</i> Species
5	<i>C. albicans</i> and <i>C. glabrata</i>
2	<i>C. albicans</i> and <i>C. krusei</i>
1	<i>C. albicans</i> and <i>C. lusitaniae</i>
1	<i>C. glabrata</i> and <i>C. guilliermondii</i>
1	<i>C. krusei</i> and <i>C. tropicalis</i>
1	<i>C. albicans</i> , <i>C. glabrata</i> and <i>C. tropicalis</i>
1	<i>C. albicans</i> , <i>C. glabrata</i> and <i>C. parapsilosis</i>

**FIGURE 1-** Associations of *Candida* species isolated from complete upper denture wearers, before the use of vinegar solution

## DISCUSSION

In the present study, daily disinfection of complete dentures with vinegar for 45 days was undertaken and the number of *Candida* genus yeasts was analyzed. The evaluation of *Candida* in saliva was chosen to investigate whether denture cleaning and disinfection could change yeast counts in oral cavity, since the saliva reflects, in general, the whole oral microbiota. Another reason for this choice was the possibility to express the results per mL, and thus to quantify the microorganisms using a more accurate method.

Although the use of the vinegar solution has reduced the *Candida* counts in the saliva of the patients, the high prevalence of *C. albicans* was still observed, being isolated 37 strains in the first saliva collection and 34 in the second one. Likewise, Geenwalt, et al.<sup>10</sup> (1998), evaluated the antimicrobial action of a fermented drink (Kombucha) with a concentration of 7 g L<sup>-1</sup> of acetic acid and did not find an inhibitory action against *C. albicans*. It is likely that the acid pH favored the adherence of *C. albicans* to the denture acrylic and then to the mucosa<sup>15,17</sup>. Bernabé, et al.<sup>3</sup> (2004) also verified no reduction in *C. albicans* counts using 0.5% of sodium hypochlorite and coconut soap as antimicrobial agents. However, as hypochlorite has an unpleasant smell and taste, the denture must be properly washed to remove the product, and its continued use can produce stains on the

denture surface, which did not occur with the use of 10% vinegar in the present study.

*C. albicans* is an opportunistic pathogen frequently isolated from oral cavity of denture wearers<sup>28</sup>, being considered the main etiological agent of the denture-induced stomatitis<sup>1,21</sup>, which is characterized by a pathological modification of the oral mucosa that covers the denture bearing tissues. It is more frequently found in patients wearing poorly cleaned and poorly fitted dentures for long periods<sup>4,25</sup>. The results of the present study showed the presence of denture-induced stomatitis in 70.8% of the studied population, confirming the high frequency of this disease in complete denture wearers.

Denture use can reduce the salivary flow, which interferes with the physiological cleaning function of the tongue and creates a favorable environment for microbial survival<sup>4</sup>. In addition to this, *Candida* cells seem to have affinity with denture acrylic<sup>23</sup>, favoring the adhesion of epithelial cells to the oral mucosa and the onset of denture stomatitis<sup>29</sup>. Denture use can thus be related to the presence of *Candida*, which can be associated with the severity of denture-induced stomatitis, as observed in this study. After use of vinegar solution, an expressive reduction in number of stomatitis cases was observed, mainly in patients with type II stomatitis, according to Newton classification.

When denture use is analyzed alone, *C. albicans* was the most common specie, representing 77% of the isolated microorganisms<sup>14</sup>. *C. albicans* was the most prevalent specie in this research, followed by *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. guilliermondii*, and *C. lusitaniae*. Similar results have been reported by Davenport<sup>7</sup> (1972) and McMullan-Vogel, et al.<sup>16</sup> (1999), who had identified *C. albicans* in the saliva of 70% and 75% of complete denture wearers with stomatitis, respectively. However, in complete denture wearers who have more predisposing factors, many others species can be isolated<sup>13</sup>. The results of the present study demonstrated a higher prevalence of other *Candida* species before the use of vinegar (*C. guilliermondii*, *C. lusitaniae* and *C. parapsilosis*). Another interesting fact was the reduction in the number of associations with two and three species after the treatment.

A significant reduction in the number of patients with unsatisfactory denture hygiene after the treatment was

observed in this study, demonstrating the efficiency of denture brushing followed by overnight immersion in the vinegar solution. Brushing associated with some kind of detergent, soap or dentifrice is the most frequently used method by removable denture wearers<sup>5</sup>. However, the effectiveness of brushing alone seems to be limited<sup>15</sup>, probably because of the existence of irregularities in the acrylic resins, which makes difficult the mechanical removal of microorganisms<sup>7,15</sup>. Therefore, in order to obtain high-quality denture cleaning, brushing and immersion in chemical agents must be associated.

In spite of the development of new techniques and materials for complete denture cleaning, patient's lack of information, low purchasing power, reduced visual accuracy and the limited manual dexterity, which are common characteristics of elderly people, impair the indication of an efficient method for denture cleaning by prosthodontists. Although the vinegar solution was not able to eliminate *C. albicans* completely, it was effective in controlling other *Candida* species, since the counts of *Candida* spp. were reduced after the treatment.

The results of this study demonstrate that the search for new alternatives for the control of *C. albicans* in complete denture wearers should persist, since the 10% vinegar solution was not able to remove efficiently this yeast, which is the main etiological agent of denture-induced stomatitis.

## CONCLUSIONS

The immersion of complete dentures in 10% vinegar solution, during the night, reduced the counts (cfu/mL) of *Candida* spp. in saliva and the presence of denture-induced stomatitis in the studied population.

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