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ORIGINAL ARTICLE

Evaluation of Several Denture Cleansers' Efficacy Against Denture-derived *Candida albicans* and Comparison with Some Antifungals' Efficacy

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ABSTRACT

Background: *One component of the oral cavity's microbiota is* Candida spp. C. albicans *can colonize the surface of an acrylic denture. Denture cleansers are a chemical approach that can inhibit the candidal growth.* ***Objective*** *: To examine the impact of some denture cleansers on denture-isolated* C. albicans *and compare them to certain antifungals.* ***Methodology****: Two sterile swab samples were obtained from each participant from 52 denture wearers. Samples were cultured on culture media to detect* Candida *growth. Sterile paper disks loaded with household chlorine, chlorhexidine digluconate, Corega tablets, salt solution, fluconazole and nystatin were tested for their antifungal effect against* Candida *by* ***disk-diffusion assay.Results:*** *48% of denture wearers have* Candida spp*. The most frequent species found in denture wearers is* C. albicans*. According to the inhibition zone diameters, the disks loaded with household chlorine showed the strongest effect on the* C. albicans *and other species of* Candida *compared with disks loaded with other denture cleansers and antifungals.* ***Conclusion:*** *The growth of oral candidiasis is facilitated by wearing dentures, and one of the most often isolated species is* Candida albicans*. The denture-cleaning household chlorine had the highest antifungal effect on* C. albicans *and other* Candida *species, even when compared with antifungals such as fluconazole and nystatin.*

INTRODUCTION

One component of the oral cavity's microbiota is *Candida spp*. [1] Under normal circumstances, oral commensal *Candida spp* can become a pathogen, making people who wear dentures more prone to developing denture stomatitis [2]. Numerous species of *Candida*, such as *C. albicans*, *C. famata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*, were isolated from the oral cavities of denture wearers[3]. *C. albicans* is a typical component of the respiratory and digestive systems' flora. It can adhere to the base of the denture and the oral mucosa and form biofilm[4], which may increase *candida* pathogenicity by improving adherence[5]. Also,the host's weak immunity contributes to its virulence [4]. Therefore, the most common cause of denture stomatitis is *C. albicans.* [6].

Cleaning dentures is an essential process that helps prevent infection and improves a patient's overall well-being, denture firmness, and general health. [7] Chemical and mechanical treatments are the two basic methods; both can also be used to preserve denture cleanliness, even though studies have not demonstrated that one strategy is superior to another [8].

Mechanical methods include using manual toothbrushes and/or vibrational-based cleaning aids such as an ultrasonic or sonic bath. Denture cleaners use chemicals; one benefit of chemical disinfectants is that they leave denture surfaces with few scratches, which prevents plaque buildup and discolouration which can be categorized into grouping according to the chemistry of their mode of action, such as oral rinses, effervescent types, mineral acid-based, enzyme-based, and bleach-based [8,9]. It has been proved that chemical approaches are more efficient than mechanical ones. The easy-to-use and efficient chemical approach can inhibit Candida's biofilm production. Therefore, they can be used instead of mechanical methods [10]. The frequency of usage of denture cleaners was linked to the amount of *Candida spp*. on dentures and the use of cleansers. Performing a daily overnight denture cleaning is an effective method of managing *Candida spp* by reducing their adherence to the denture base [11], leading to a reduction in *C. albicans* colony number, which improves the denture wearer's health[12].

This study aims to examine the impact of some denture cleansers on denture-isolated *C. albicans* and compare them to certain antifungals.

METHODOLOGY

Participants

Fifty-two denture wearers (twenty-seven wearing upper and lower partial dentures and twenty-five wearing complete dentures) came to outpatient dental clinics for various reasons. Their ages ranged from 20 - 80 years old, with 22 females and 30 males. Each Participant filled out a questionnaire about how to clean their dentures, what kind of cleanser to use, taking off their dentures while sleeping, whether they had mouth inflammation, whether dentures are cleaned daily, whether they had any immunosuppressive diseases, and if they are smoking. As a control group, fifty Participants without dentures had samples obtained from their oral cavity.

Sample collection

Two sterile –swab samples were obtained from each participant wearing dentures: one was taken from the base of the denature and the other from the gum line beneath it. Those without dentures, control, also had a sample obtained from their oral cavity. Separate cultures of these samples were made on a plate of Sabouraud agar, and they were incubated at 37 degrees for 48 hours(aerobically), or until yeast colonies appeared.

Identification of *Candida spp*

A set of conventional assays, including formation germ tube, colony phenotypic appearance, fermentation, urea hydrolysis, and C and N assimilation, were used to identify the different species of *Candida*. The diagnosis was confirmed using the colour and form of the colony on CHROM agar candida.

Selection of denture cleansers

A set of denture cleansers was selected according to how study participants cleaned their dentures. Household chlorine (deionized water and sodium hypochlorite with concentration 100%,60%, and 40%), mouthwash (0.12% chlorhexidine digluconate and 0.05% sodium fluoride), Corega denture cleanser tablets (sodium bicarbonate and sodium perborate), and solution of salt (NaCl)with concentration 80%,60%, and 40% were the selected cleanser.

Disk-diffusion assay

Sabouraud dextrose agar plates were prepared, and then 0.1 ml of a suspension of *Candida spp* was spread on the plates. Disks of filter paper with a 0.6 mm diameter were sterilized by autoclave and submerged in a denture cleanser solution. Disks were separately put on the agar surface and pressed slightly on it, then they were incubated for 24 hours at 37C°. Then it was measured the diameter of the inhibitory zone surrounding each disk in millimeters. The assay follows CLSI protocols [13].

Comparison with antifungal drugs

Candida albicans isolates were evaluated against fluconazole and nystatin by disk diffusion assay using disks of (25µg) fluconazole and (400µg) nystatin (nystatin disks were prepared by soaking in a 20 µl nystatin 100 000 IU/ml mixture[14] to obtain 400 µg nystatin on each disk diffusion[15] (CLSI, 2009) [16].*C.albicans* suspensions were spread onto Sabouraud dextrose agar plates and disks were put on the surface. After the incubation period, the inhibition zone diameters were measured in millimeters. The susceptibility of isolates was categorized according to inhibition zone diameter [17,18]. Then, A comparison of the diameters of *Candida* inhibition zones generated by denture cleansers and those by antifungals was made.

RESULTS

Candida spp was isolated from the denture base and gum of twenty-five (48%) participants who wore dentures (50 isolates). *C. albicans* was isolated from the oral cavity of eight (16%) participants who did not use dentures (control group). The most frequent species found in denture wearers' dentures is *Candida albicans* and the only one isolated from non-denture wearers, as shown in Table 1.

According to the questionnaire, 32% of participants who had *Candida* isolated from their dentures exhibited denture stomatitis, whereas 11% did not have *Candida* isolated from their dentures did not exhibit any symptoms.70% of participants did not wear their dentures to sleep and 63% of them cleaned their denture daily and there was no *Candida spp* was found to be on their dentures. While *Candida* colonization was not significantly impacted by smoking, diabetes, and the type of denture. Notably, though, no isolate of *Candida spp* was recovered from the dentures of diabetics who cleaned their denture with household chlorine Table 2.

Table 1. Isolated *Candida spp* from denture wearer and control group

Participants	*Candida spp.no(%)*							
	C.albicans	*C.famata*	*C.kefyr*	*C.trpoicalis*	*C.glabrata*	*C.krusi*	*C.guilliermondii*	total
Denture wearers	12 (48)	5 (20)	2 (8)	2 (8)	1(4)	2 (8)	1(4)	25
Control group	8 (100)							8

Table 2: Predisposing factors that promote growth of *Candida spp* on dentures.

Predisposing factors	Number of participants with positive for Candida (%)	Number of Participants with negative for Candida (%)
Denture cleaning		
Daily	11/25(44)	17/27 (63)
Not daily	14/25(56)	10/27 (37)
Type of deanture		
Complete	12/25 (48)	14/27 (52)
Partial	13/25 (52)	13/27 (48)
Denture removal at sleeping		
Yes	11/25(44)	19/27 (70)
No	14/25(56)	8/27 (30)
Diabetes mellitus		
Yes	4 /25(16)	5/27 (19)
No	21/25 (84)	22/27 (81)
Diabetic patients who use of household Chlorine		
Yes	2/25 (50)	5/27 (100)
No	2/25 (50)	0/27 (0)
Smoking		
Yes	4/25 (16)	3/27 (11)
No	21/25 (84)	24/27 (89)

Table 3 shows that 48% of participants who used household chlorine to clean their dentures had isolates clear of *Candida*.60% of participants who used toothpaste had isolates positive for *Candida*. There was no difference between the two groups when salt and Corega tablets were used.

According to the diameters of the inhibition zone, the disks loaded with household chlorine showed the strongest effect on the *C. albicans* and non *C. albicans spp* at different concentrations that were used when they were compared with disks loaded with other denture cleanser. Meanwhile, disks of dental sterilizing tablets (Corega tablet) had the least impact on *Candida spp*. On the other hand, there was no effect of the disks loaded with a salt solution at any concentration on *Candida spp,* as shown in Figure 1,2.

Figure 3 shows that even at all concentrations, household chlorine has a greater effect on *C.albicans* than antifungals, with a significant difference at 100% concentration, as indicated in Table 4.

On the other hand, Antifungals had a greater effect on *C.albicans* than the other denture cleansers, with significant differences, as show in Table 4.

Table 3: Denture cleanser types and their impact on denture *Candida spp* growth

Type of denture cleanser	Number of Participants with positive for Candida (%)	Number of Participants with negative for Candida(%)
Salt Solution (NaCl)	7/25 (28)	7/27(26)
Household chlorine	8/25(32)	13/27(48)
Corega tablets	2/25(8)	1/27 (4)
Toothbrush + toothpaste	15/25(60)	14/27(52)
Water only	4/25(16)	
Others	2/25(8)	

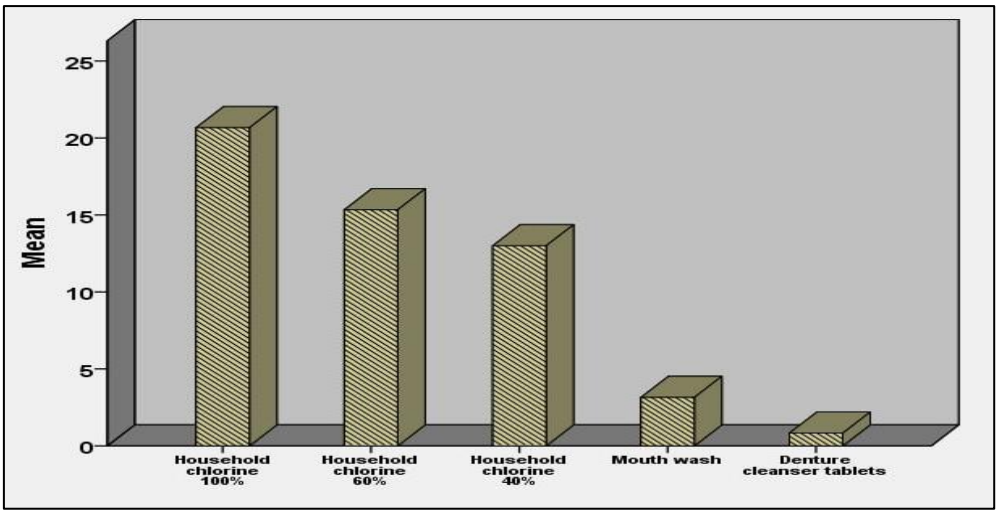

Fig. 1: Denture cleansers' effects on *C. albicans*.

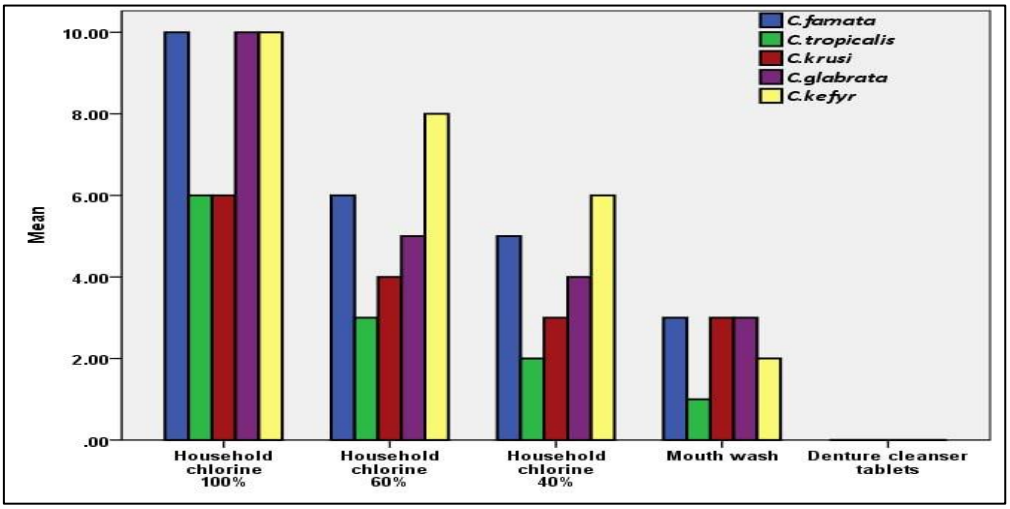

Fig. 2: Denture cleansers' effects on *candida spp*.

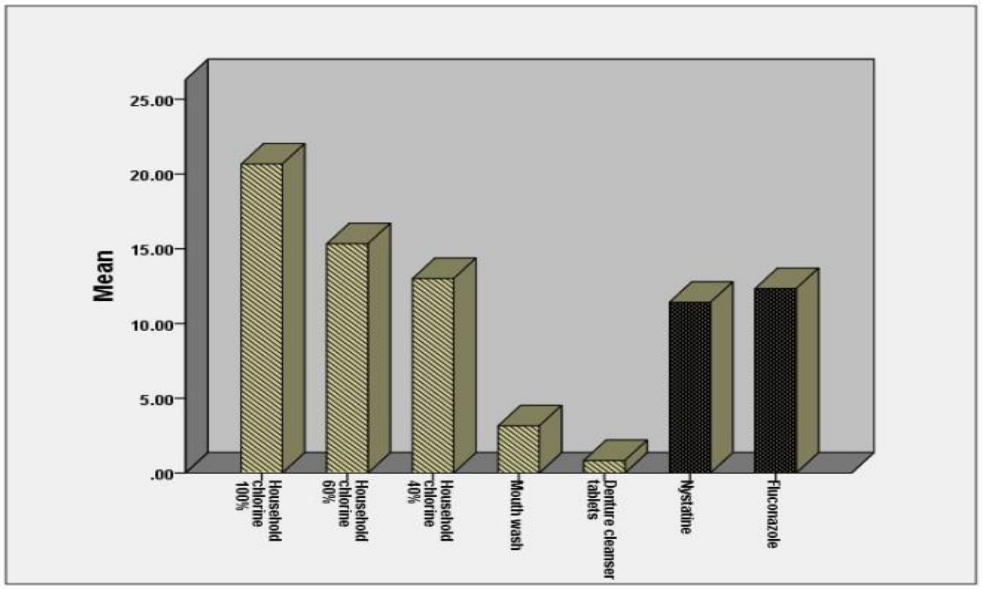

Fig. 3: Comparison of the effects of denture cleansers and antifungals on *C.albicans* based on diameters of inhibition zones

Table 4: Significant difference between the effect of denture cleansers and antifungals on *C. albicans* based on inhibition zone diameters.

		P-value
Candida C.albicans	Household chlorine100% V.S Nystatine&Fluconazole	0.0004*
	Household chlorine 60% V.S Nystatine& Fluconazole	0.0718
	Household chlorine 40% V.S Nystatine& Fluconazole	0.5105
	Mouth wash(chlorhexidine) V.S Nystatine& Fluconazole	0.0001*
	Corega tablets V.S Nystatine& Fluconazole	0.0000*

*The difference is significant at level (0.01)

DISCUSSION

Prolonged denture wearing has been regarded as the primary risk factor for oral cavity colonization by *Candida spp*. [19]. In this study, it was established that wearing denture is predisposing factor for Candida colonization, which agreed with the findings of Benyounes *et al.,* who found that the number and growth of *Candida spp* increased significantly through wearing acrylic dentures [20].

Agreement with current findings, *C. albicans* was the commonest isolated species from dentures compared to prevalence of other b *as C. glabata*, *C. tropicalis* [21], *C. guilliermondii, C. famata*[22] and *C. krusei* [23] due to *C. albicans* possesses highly virulence factors [24]. *C. albicans* is linked to the onset of denture stomatitis [25].

Several predisposing factors can impact *Candida spp* colonization in the oral cavity and base of dentures, such as poor hygiene like wearing dentures at night [26] and intermittent cleaning because it was found that daily cleaning of dentures was associated with controlling the growth of *Candida spp* on denture[27], a relationship was also found between smoking and increased *Candida* colonization on dentures due to its role in increasing formation of *Candida* biofilm and stimulation of *Candida* growth [28]. However, these findings are not consistent with the results of this study; the reason may be due to the small number of smokers participating in this study. Diabetes mellitus contributes to oral candidiasis, especially by *C. albicans* of denture wearers compared to their peers without diabetes[29].

Our results show that household chlorine had the highest diameters of inhibition zone against *Candida albicans* and other species compared to denture cleansers that were used; in 48% of participants who used it, even diabetic patients who used chlorine to clean the dentures, there was no oral *candida spp* detected that may due to hypochlorite solution can reducing denture biofilm [30] because it can kill fungi, bacteria and dissolve organic material which associated with its alkaline nature, which has role in dissolving the cells embedded in biofilm on denture material [31].

Despite many prior researches showing chlorhexidine's ability to reduce colonization of *Candida spp* on dentures[32] because it inhibits fungal growth, formation of biofilm, and even survival of biofilm cells [33], the current findings revealed that the diameters of *Candida spp* inhibition zone were less than those of household chlorine.

Corega usually contains sodium bicarbonate and sodium perborate. The alkaline peroxide solution forms in its watery solution, and the sodium perborate dissociates. The role of peroxide solution is to remove the debris from the denture by releasing oxygen and degrading biofilm proteins by producing oxygen-free radicals with antimicrobial activity [34]. Findings of the current study showed that disks loaded with a solution of Corega tablets affected *C.albicans* (Despite having less of an impact than another denture cleanser that was used) but not on other species of *Candida*, which validates findings of Geduk et al., who found highly antifungal effect of corega solution [35]. It also supported the findings of Ghazal et al., who discovered that the Corega tablets were more effective than Chlorhexidine at eliminating all viable *C. albicans* cells from the surface of acrylic bars (after treatment with them in vitro) at a 100% rat[36].

C. albicans and other *Candida spp*, such as *C. glabrata,* can be classified as osmotolerant fungi. *C. albicans* is the most salt-tolerant and can grow in high external NaCl concentrations (halophilic) [37]. So, salt (NaCl) solution as a denture cleanser has the lowest efficiency in eradicating *Candida* colonies[38]. In agreement with the current study's findings, the salt solution did not show any effect on any species of *Candida*.

Denture stomatitis is usually treated with fluconazole and nystatin [39]. Many studies have shown the efficacy and effectiveness of incorporating nystatin into several tissue conditioners for treating denture wearers with denture stomatitis [40]

Also, it was found that when soft denture liners were incorporated with fluconazole to treat *C. albicans* biofilms, this led to a decrease in the cell count of *C. albicans* [41]. However, Figueiral et al. previously found that fluconazole had a good short-term denture stomatitis treatment efficiency, but may have contributed to the long-term development of fluconazole resistance in *Candida albicans*[42].However, if care is neglected, using antibiotics does not prevent the colonization of *Candida* on the denture but helps ease the patient's symptoms[40]. So, mechanical and chemical

methods for cleaning the denture may be recommended as a supplement or substitute for antifungal drugs[43]. The present findings demonstrated that household chlorine (100%) had a stronger effect on *C.albicans* than antifungals.

CONCLUSION

The growth of oral candidiasis is facilitated by wearing dentures, and one of the most often isolated species is *Candida albicans*. The denture-cleaning household chlorine had the highest antifungal effect on *C.albicans* and other Candida species, even when compared with antifungals such as fluconazole and nystatin.

This study has received ethical approval from the University of Basrah's College of Medicine's Ethical Committee. [4/473, 28/11/2021]

Informed consent was obtained in accordance with institutional guidelines.

Conflict of Interest

There are no significant conflicts of interest among the authors relevant to this research subject

Financial Disclosures

There are no specific financial interests, relationships, or affiliations relevant to the subject matter of the manuscript.

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