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ORIGINAL RESEARCH

Isolation of *Candida* Species from the Oral Cavity and Fingertips of Complete Denture Wearers

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ABSTRACT

Background: Wearing a dental prosthesis is known to increase oral candidal colonization and predispose the wearer to oral candidosis. Denture wearers frequently use fingers to take the prosthesis out of their mouth. Oral *Candida*, if present may contaminate wearer's finger. The objective of this study was to investigate the simultaneous candidal colonization of oral cavity and fingertips of complete denture wearers.

Materials and methods: A total of 25 apparently healthy male subjects who had worn complete dentures for at least 1 year were selected. Information about each patient's denture age, denture hygiene, handling, and wearing habits, and hand washing habits after denture handling were be obtained. Intraoral examination of all the patients was done. For microbiological examination samples were collected from the fingertip and oral rinse of each patient. *Candida* species were identified with use of germ tube test and commercially available yeast identification system. Data was statistically analyzed. Significance was set at $p < 0.05$.

Results: It was found that frequency of hand washing, denture handling and denture stomatitis with respect to fingertip candidal isolation was not statistically significant. But poor denture hygiene and denture stomatitis with respect to oral candidal colonization was statistically significant.

Conclusion: Denture wearers with oral *Candida* had a higher prevalence of *Candida* contamination on their fingers. Patients with removable prostheses should be informed about the importance of proper prosthesis and personal hygiene and the possibility of microbial contamination of the hands and other parts of the body.

Keywords: *Candida*, Denture stomatitis, Denture wearer.

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INTRODUCTION

Candida species are normal oral commensals present in 30 to 70% healthy persons. They commonly colonize the vagina and skin.[1] Wearing a dental prosthesis is known to increase oral *Candida* colonization and to predispose to oral candidosis.[1,2] This has been explained by the high affinity of *Candida* species to adhere by electrostatic interaction and subsequently colonize on rough surface of denture acrylic resin material.[3,4]

Epidemiologically yeasts are demonstrated in 47% of individuals with no clinical signs and 80% individuals with denture stomatitis. On the basis of the frequencies of yeast isolation, yeast is main etiologic factor in denture stomatitis.[5] Wearing of prosthesis is widely regarded as a local etiological factor denture stomatitis.[6] Bacterial and yeast plaque on dentures may cause infections, and that oral *Candida* infection in debilitated patients may be the spearhead of a systemic and fatal *Candida* infection.[7]

Removable denture wearers frequently use their fingers to take their prosthesis out of their mouth, either to cleanse or simply check them. Oral *Candida* if present, may contaminate the fingers.[1] The simultaneous candidal colonization of the oral cavity and finger tip of a complete denture wearer has not been investigated. So purpose of this study was to know the simultaneous *Candida* colonization of the oral cavity and fingers of complete denture wearers.

MATERIALS AND METHODS

For examination of the oral cavity mouth mirror, probe, gloves, mouth mask, tray and cotton were used. Sterile disposable plastic container, filter paper, spirit lamp, sterile 5 ml glass pipette, Sabourauds' dextrose agar plate and phosphate buffered saline were used for collection of oral rinse, fingertip sample and culture. Micropipette, Incubator (Kumar industries, Mumbai) Colony counter (Kumar industries, Mumbai) were used for incubation.

For Gram staining spirit lamp, microscopic slide, Grams stain and microscope were used. Germ tube test was done with spirit lamp, microscopic slide, wire loop, human serum, Grams stain and microscope. KB006 – *Candida* identification kit was used for species identification.

Selection of Subjects

A total of 25 apparently healthy male subjects who had worn complete dentures for at least 1 year were selected from the department of Prosthodontics. Subjects selected had ages between 50 and 70 years with the mean age of 59.04 years and denture age between 2 and 11 years with mean denture age of 4.76 years.

Subjects who were on antifungal and antiseptic mouth washes, long-term antibiotic therapy, medication known to predispose them to oral candidiasis, having a medical history that revealed any diseases or medical condition that predisposed them to oral candidiasis, medical history that revealed any disease or medical condition such as diabetes mellitus, immunocompromised patient were excluded from the study.

Clinical Study Design

A thorough medical and dental history was taken of each subject. Information about each subjects age, denture age, denture hygiene, handlings and wearing habits, and hand washing habits after handling of dentures were obtained through a structured interview conducted immediately prior to the clinical examination.

Intraoral Examination

Intraoral examination of all subjects was conducted after the subjects removed their dentures. A careful intraoral examination was done, if denture stomatitis encountered, it was be classified according to Newton's criteria.[8]

Assessment of Denture Cleanliness

Denture cleanliness of each subject was assessed using erythrosine dye (planksee) to disclose plaque on the denture. After removal of denture from the mouth the denture was rinsed under running water. Then the denture was applied with an erythrosine dye (planksee) with help of cotton bud and left for 1 minute, and then denture was rinsed to remove excess stain. The disclosed stainable plaque on the palatal fitting was recorded and the subjects were divided into three groups by using Butdz-Jorgensen's index of denture cleanliness.[9]

Collection of Samples for *Candida* Analysis

Fingertips Samples

Each subject was asked to press the fingertips of both of his hands on a plate of Sabouraud's agar for 1 minute. Fingertip sampling was performed after case history and before touching his dentures.

Oral Rinse Samples

Each subject was supplied with 10 ml of sterile phosphate buffered saline in a sterile container and requested to rinse the mouth thoroughly in the presence of the clinician for 60 seconds. The subject then returned the mouth rinse to the sterile container which was sent to laboratory for microbiologic analysis.

Storage of Samples

Immediately after collection the samples they were numbered and then transferred to the Department of Microbiology, S. Nijalingappa Medical College and Hospital, Bagalkot for further evaluation.

Incubation of *Candida*

Fingertip Sample

Fingertip sample Sabouraud's agar plate was incubated aerobically for 48 to 72 hours at 37°C, the numbers of colony forming units were identified using colony counter and colony forming unit per millimeter of Sabouraud's agar plate Figure 1.

Oral Rinse Sample

100 ml aliquot was taken from oral rinse centrifuged for 10 minutes then was collected in sterile container and plated in Sabouraud's dextrose agar. After 48 to 72 hours incubation at 37°C the number of colony forming units, were identified using colony counter and colony forming units per milliliter of rinse were determined.

Preparation of Sabouraud's Agar Plate

Sabouraud's agar plate was prepared by dissolving peptone, agar in water and adjusting Ph to 5.4, then autoclave at 115° for 15 minutes, 20 ml amounts was dispensed into petri dishes.

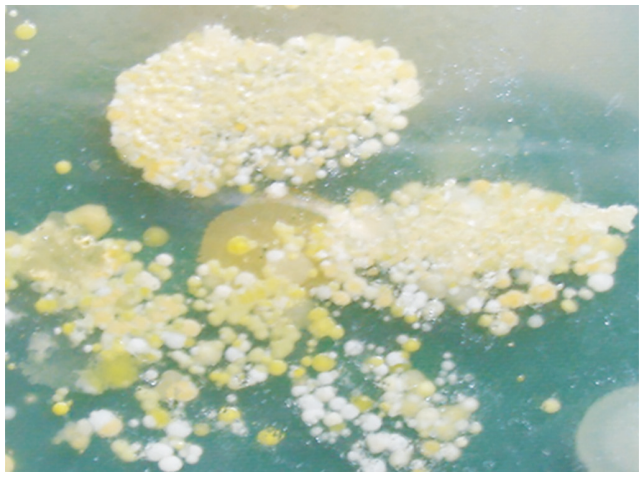

Fig. 1: Colonies of *Candida*

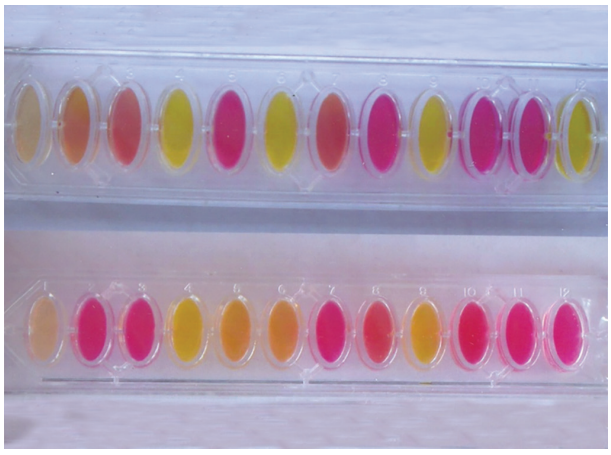

Fig. 2: Different biochemical test indicated by chromogenic changes in identification kit

Species Identification

It is done by two methods:

1. *Candida* identification kit.
2. Germ tube test.

CANDIDA IDENTIFICATION KIT

KB006 is a standardized test system that can be used for identification of *Candida* species. It can also be used for validating known laboratory strains. The complete list of organisms that can be identified with this system is given in the identification index provided with the kit (Fig. 2).

Principle

Each KB006 kit is a standardized colorimetric identification system utilizing twelve conventional biochemical tests. The tests are based on the principle of pH change and substrate utilization on incubation; organisms undergo metabolic changes which are indicated by a spontaneous color change in the media.

Preparation of Inoculums

KB006 cannot be used directly on clinical specimens. The organisms to be identified were first isolated and purified. Only pure cultures were used. The organisms to be identified were isolated on a common medium like Sabourauds dextrose agar. The inoculum was prepared by picking 2 to 4 well isolated colonies and homogenous suspension was made in 2 to 3 ml sterile saline. The density of the suspension was adjusted to 0.50D at 620 nm. The kit was opened aseptically by peeling off the sealing foil. Then each well was inoculated with 50 µl of above inoculums using surface inoculation method. The inoculums were incubated at a temperature of 22.5°C ± 2.5°C for duration of 24 to 48 hours.

Germ Tube Test

The presence of *Candida albicans* was confirmed by germ tube test. For the germ tube test, a single colony was lightly touched with a loop or Pasteur pipette, the excess inoculum was removed and then the yeast cells were emulsified in 0.5 ml of serum in small test tube with a loose cotton wool plug. It was incubated at 37°C in a water bath for 24 hours. The above inoculums were mounted on a mounting slide in oil immersion and examined using compound microscope at 100x magnification. It was examined carefully to ensure that the parent cell wall and the germ tube are one continuous cell and that a cell wall does not separate the two as in *Candida tropicalis*.

The presumptive identification of *Candida albicans* was usually sufficient but where absolute certainty was required, the inability of *Candida stellatoidea* to assimilate sucrose was diagnostic. Identification of germ tube negative yeast may also be necessary on occasions, especially to determine the role or possible source of the isolate or potential drug resistance.

STATISTICAL ANALYSIS

The data was collected and recorded and analyzed statistically by chi-square test, t -test and Mann-Whitney's test.

RESULTS

(Table 1A) shows Pair wise comparison of denture cleanliness with respect to colony forming units (CFU/ml) for oral *Candida* species (Table 1B). Analyzing the data with t-test, it was found that statistically significant relationship exists between the groups (Table 2). With respect to the Relationship of fingertip *Candida* isolation to frequency of denture handling using there is a difference between the two groups, but no of subjects with denture handling frequency of 1 to 3 times are more when compared 4 times and above. It was found that, there is no statistically significant relationship between the two groups (1 to 3 times and 4 times and above) with p-value of 0.0076 (Table 3). It was found that, there is no statistically significant relationship between fingertip *Candida* isolation with respect to hand washing the two groups with p-value of 0.4274 (Table 4). It was found that, there is statistically significant relationship exists between oral *Candida* isolation to denture stomatitis the two groups (denture stomatitis and normal palatal mucosa) with p-value of 7.9102.

DISCUSSION

In the present study, a significant proportion of the denture wearers who had oral *Candida* (64%) also had *Candida*

colonized fingertips (62.5%) indicating that their hand may have been contaminated with the oral microbes during denture handling. Two subjects with *Candida* free oral cavity had *Candida* colonized finger tips suggests that there were other sources of contamination.

The isolation of two species *Candida parapsilosis* and *Candida rugosa*, from only the fingertips supports the idea that other sources of hand contamination were present.[1] A study conducted by Huang and others showed that the hands of 29% of hospital personnel were contaminated with *Candida* species (commonly *Candida albicans* and *Candida parapsilosis*), and the species were not acquired from a common source.[10] *Candida rugosa* has been isolated from human feces and bovine droppings and is the second most frequently isolated yeast from bovine mastitis.[11]

In this study 5 subjects showed excellent denture cleanliness with mean value of 0.000 and 16 subjects showed fair denture cleanliness with mean value of 188.0625 and 4 subjects showed poor denture cleanliness with mean value of 327.000. The *Candida* colonization was more in poor denture hygiene when compared to fair and there was no colonization in excellent denture hygiene. This study demonstrated a strong association between poor denture hygiene and oral candidal colonization. These findings are similar to studies conducted by Azmi and Grimoud,[1,12] and tends support to research that plaque accumulation on the denture surface may create an appropriate environment for yeast growth.[13]

There was no statistically significant relationship between *Candida* colonization to the frequency of denture cleansing. This was in agreement with other studies.[1,9,14] Similarly, there was no statistically significant relationship between oral *Candida* colonization to the denture age. This was in agreement with other studies.[1,15,16]

Table 1A: Pair-wise comparison of denture cleanliness with respect to colony forming units (CFU/ml) for oral *Candida* species

Denture cleanliness (No. of subjects)	*CFU (SD)*
Excellent (n = 5)	0 (0.0)
Fair (n = 16)	3009 (115.6)
Poor (n = 4)	1308 (40.8)
Total (n = 25)	13

CFU: Colony forming units; SD: Standard deviation

Table 1B: Analyzing the data with t-test

Denture cleanliness	*Mean*	*SD*	*t-value*	*p-value*	*Significant*
Excellent	0.0000	0.0000	–3.5708	0.0020	S
Fair	188.0625	115.6935			
Excellent	0.0000	0.0000	–18.2501	0.0000	S
Poor	327.0000	40.8003			
Fair	188.0625	115.6935	–2.3246	0.0320	S
Poor	327.0000	40.8003			

SD: Standard deviation; S: Significant

Table 2: Relationship of fingertip *Candida* isolation to frequency of denture handling by chi-square test

Frequency denture handling (n = No. of subjects)	*+ve fingertip isolation*	*-ve fingertip isolation*	*p-value*	*Significance*
1 to 3 times (n = 21)	11 (52.3)	10 (47.6)	0.0076	NS
4 and above (n = 4)	2 (50)	2 (50)		
Total (n = 25)				

NS: Not significant

Table 3: Relationship of fingertip *Candidal* isolation with respect to hand washing by chi-square test

Hand wash (n = No. of subjects)	*Positive finger tip (%)*	*Negative fingertip (%)*	*p-value*	*Significance*
With hand wash	7 (47)	8 (53)	0.4274	NS
Without hand wash	6 (60)	4 (40)		
Total (n = 25)	13	12		

NS: Not significant

Table 4: Relationship of oral *Candidal* isolation to denture stomatitis by chi-square test

Denture stomatitis (n = No. of subjects)	*+ve oral Candida (%)*	*–ve oral Candida (%)*	*p-value*	*Significance*
Denture stomatitis (n = 9)	9 (100)	0 (0)	7.9102	S
Normal (n = 16)	7 (44)	9 (66)		
Total (n = 25)	17	8		

S: Significant

The results of the current study indicate that denture wearers who did not wash their hands after denture handling or who handled their dentures frequently per day had a relatively higher proportion of fingertip Candidal isolation than those who washed their hands and handled their dentures less frequently. However, the difference was not statistically significant.

There was a significant relationship between denture stomatitis and oral Candidal isolation. The findings that *Candida* species were isolated from the oral cavity of 100% of subjects with denture stomatitis may indicate possible role of *Candida* species. This was in agreement with Ramage study.[17] The findings that 32% of the complete denture wearers in this study had Newton type 1 denture stomatitis is less than other studies (56%).

The present study showed that there is no statistically significant relationship between finger tip *Candida* colonization and denture stomatitis. There is more *Candida* colonization in denture stomatitis when compared to normal palatal mucosa that suggestive of possible role of *Candida* species.

The current investigation showed *Candida albicans* was most frequently isolated from the finger tip and oral rinse sample, that suggestive possible role in denture stomatitis. This was in agreement with other studies Barbeau Daniluk.[18,19]

Candida isolates in the current study were identified with the hi-media *candida* kit KB 006. However, it is not clear whether rare isolates recovered from the subjects (*Candida rugosa*) came from an external source, such as animals, or constituted a part of the permanent yeast flora in this subjects.[1]

Additional studies also identified species at the strain level and sample. Other possible source of contamination (such as gastrointestinal tract and vagina, in addition to the oral cavity and surrounding environment) are needed to define precisely the source of hand candidal contamination. The clinical significance of candidal finger contamination should be determined, and the possibility that contaminated finger will act as reservoir of infection and reinfect the oral cavity that has been treated with antifungal therapy should be addressed.

Although the current investigation was not designed as an epidemiologic study and did not aim at studying oral *Candida* in denture wearers, it was noticed that the candidal recovery rate of the study population (64%), similar to study conducted by Azmi.[1]

CONCLUSION

It was found that relation between frequency of oral and fingertip candidal isolation was statistically not significant. Similarly frequency of hand washing, denture handling and denture stomatitis with respect to fingertip candidal isolation was also not statistically significant. But poor denture hygiene and denture stomatitis with respect to oral candidal colonization was statistically significant.

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