Original Article

Evaluation of microbial contamination in removable dental prosthesis at different time of usage

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Abstract

Aim: The current study evaluates and compares the percentage distribution of different microorganisms according to their strains and occurrence among the three studied groups.

Method and Methodology: Sample of 30 removable dental prosthesis wearing patients was selected: wearing either complete dentures or partial dentures and without any significant medical history or on prescription medication for the past 3–6 months. Samples were obtained in three subcategories based on the duration of prostheses worn by the patient. A sterile swab made up of cotton moistened with phosphate buffer saline (PBS) was scrubbed on the dental prosthesis at the fitting surfaces and the denture-bearing area of the oral cavity. Within two hours, the collected swab sample was infused in the sterile tube containing 1 ml of 0.84% PBS solution maintained at pH 7–7.2 and sent for microbiological analysis. The samples were then inoculated into different medias. Microbial growth was checked after incubating the culture plates for 48 h at 37°C. Microorganisms were recognized and counted by calibrated colony counter. Gram's stain was used to stain the colony smear and biochemical tests such as coagulase, catalase, oxidase, sugar fermentation with acid and gas production (triple sugar iron), methyl red test, test for indole production, hydrogen sulphide (H_2S) production, citrate utilization, urease test, germ tube tests were performed.

Statistical Analysis: Descriptive statistics included calculation of means and standard deviation using multivariate analysis. All values were considered statistically significant for a value of $P < 0.05$.

Results: *Streptococcus species, Coagulase-negative staphylococcus, Staphylococcus aureus, Candida albicans and Klebsiella pneumoniae* showed the maximum positive culture among the secluded microorganisms in all three groups.

Conclusion: A progressive increase in the microbial contamination was directly proportional to the duration of removable prosthesis usage.

Keywords: Dental prosthesis, microbial contamination, microbial count

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INTRODUCTION

The diversity of oral microflora and its role has been evident since the time when Antonie van Leeuwenhoek first examined the microbiome of dental plaque in 1700s.[1]

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Since then, the importance of oral microflora in oral cavity has been a matter of research. Oral health reflects one's general health, affecting the ability of an individual to eat and speak, and contributes to a sense of confidence and well-being. Oral health status declines with age, and as a result, the need for removable prosthesis increases. The factors aggravating the degradation process of the oral tissues in old age could be lowered resistance to disease, impaired host defence impaired quality and quantity of saliva to name a few. Loss of teeth in the elderly is also regarded as a major factor of apprehension, affecting the individual's health. Also, microbial contamination of the dental prosthesis occurs due to neglect, lack of aseptic condition and/or lack of knowledge about denture care and maintenance.

Oral microflora changes quantitatively and qualitatively with age, presence or absence of diseases, presence or absence of teeth and other surfaces to which they can get attached.[2] Some of the commonly identified microorganism species are *Streptococcus, Staphylococcus, E. coli, Pseudomonas species, Klebsiella, pneumoniae, Candida species*, etc. The resident microflora plays an active role in the maintenance of the healthy state by contributing to the host defences and preventing colonization by exogenous microorganisms.[2]

These microorganisms are highly diverse containing about 700 bacterial and fungal species of which more than half have not yet been isolated.[3] Diseases can be a consequence of disruption of these resident microflorae. Wearing removable dental prosthesis may disrupt the oral microflora leading to the development of a particular condition such as angular cheilitis, stomatitis or denture-associated stomatitis, traumatic ulcers, denture irritation hyperplasia, etc. Denture-induced stomatitis is also a recognized clinical challenge. The responsible microorganism has not been delineated.[4]

Different studies have suggested that oral bacteria may be risk factors for many of the prevalent systemic diseases. Despite several advancements in preventative and curative dentistry, a constant concern in elderly populations is the high rate of edentulism, which is interrelated to an escalating prevalence of periodontal disease and caries. The most frequent rehabilitation for total or partial loss recovery is partial or complete dentures. Rehabilitative treatment works best when patients are aware of proper prosthesis use and cleanliness. Good denture hygiene can be a preventive measure to avoid oral mucosal infections. Many researchers have conducted many studies to appreciate the contamination of the oral cavity as in pre- and post-denture insertion.[5] The current study evaluated the microbial contamination of removable dental prosthesis with the intention of assessing microbial contamination at different time interval usage of prostheses.

MATERIALS AND METHODS

The study design

Ethical approval was obtained (approved ethical No. 2020-21/047). The current cross-sectional study was conducted on 30 randomly selected patients using removable dental prostheses. The duration of these removable prostheses in the patients' mouth was at different intervals of time period.

Inclusion criteria

- Patients wearing removable dentures: either complete dentures or partial dentures.
- Age range of the patients: between 45 and 80 years.

Exclusion criteria

- Patients suffering from systemic disease.
- Patients on prescription medications for the last 3–6 months.

Study procedure

Selection of the patient was done according to the exclusion and inclusion criteria. Samples were obtained in three subcategories:

- Group I: Day 1 of denture placement.
- Group II: Patients wearing removable dental prosthesis from past 1 month.
- Group III: Patients wearing removable dental prosthesis from past 3 months.

Under strict aseptic measures, swab method was employed to collect the samples. A sterile swab moistened with PBS was scrubbed on the dental prosthesis at the fitting surfaces and the denture-bearing area of the oral cavity. Within two hours, the collected swab sample was infused in the sterile tube containing 1 ml of 0.84% PBS solution maintained at pH 7–7.2 and sent for microbiological analysis at microbiology laboratory.

A uniform distribution of microbes was achieved by vigorous agitation of the collected swab sample. The PBS was then inoculated onto the Blood agar and MacConkey agar plates with the help of spread plate technique. A bent glass rod (spreader) was used to inoculate, and 0.1 ml of PBS was placed in the centre of the plate using a sterile pipette. The spreader was placed in contact with the inoculum of the culture plate and was homogeneously spread over the plate having even pressure.

Microbial growth was checked after incubating the culture plates for 48 h at 37°C. Microorganisms were recognized and counted by calibrated colony counter [Figures 1 and 2]. The data were tabulated as CFU/μl. Gram's stain was used to stain the colony smear, and biochemical tests such as coagulase, catalase, oxidase, sugar fermentation with acid and gas production (triple sugar iron), methyl red test, test for indole production, H_2S production, citrate utilization, urease test, germ tube test were performed from isolated Candida colonies to authenticate *C. albicans*.

Statistical Analysis: Data were entered into Microsoft Excel spreadsheet and checked for any discrepancies. The data was analysed by SPSS (21.0 version). Chi square test was used for categorical variables. The level of statistical significance was set at *P* value less than 0.05.

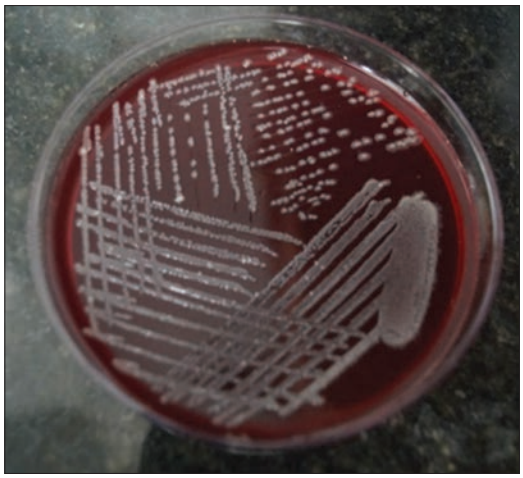

Figure 1: MacConkey agar culture plate growing the most commonly found bacteria in dental prosthesis

RESULT

The colony counts were statistically significant between all three groups. *Staphylococcus aureus* and *Candida albicans* showed the maximum positive culture among the secluded microorganisms in all three groups. *Streptococcus species* also showed a significant increase from the day of insertion to third month of usage of removable prosthesis.

DISCUSSION

Increasing awareness about modern dental treatments has urged people to replace the missing teeth with different types of dentures which restores the function of the tooth as well as maintains related structures such as muscle tone and movements. The removable partial dentures which are easily removed by patients are easily susceptible to contamination due to various environmental factors in the oral cavity.

Kareem SA *et al*.[4] conducted a study to evaluate microbial changes in oral cavity of newly edentulous patients, [Table 1 and Figure 3] before and after insertion of the dental prosthesis. The study included 28 newly edentulous patients with age range between 40 and 80 years. Saliva samples were collected at two intervals: before making the primary impression and one-month of functional use of complete dentures. Microbiologically assessment concluded that although denture can serve as a colonization site for various microorganisms within short period of denture use, good oral hygiene interfered with the microorganism growth.[6]

Nair VV *et al*.[1] conducted a similar study to evaluate the microbial infectivity of removable dental prosthesis and allocation of microorganism growth between three completely different groups. The comparative evaluation of the microbial contamination on a removable prosthesis revealed a progressive increase in microbial infectivity with the increase in time of the dental prosthesis usage. This confirmed that removable dental prostheses act as source of microbial contamination that harbours biofilm of mixed species of microbes. In the current study, *S. aureus* and *Eubacteria* species were most frequent and maximum in number among the three groups. *C. albicans* also showed its presence as the interval of usage of prostheses increases. *C. albicans* were found from the removable dental prosthesis which was being used for the past six months as compared with one month of usage. This study was comparable to the current study conducted on 30 samples at three different time intervals of usage of removable dental prosthesis which revealed a significant growth in microorganisms as the duration of usage of removable dental prosthesis increased [Table 2 and Figures 4]. *Staphylococcus aureus and C. albicans* were the highest positive culture among the isolated microorganisms in all three groups and also showed a significant difference between group 1 and group 2.[7]

In a study done by Tanaya *et al*.[2], it was found that significant difference was found in colony count in their study as well as colonizing microorganisms were found. According to time, this difference was a crucial component in the influence of RPD on oral health of denture-wearing patients [Table 3 and Figure 5]. Whereas age group was not a major factor influencing microbial health in removable denture wearing patients. Health of oral tissues had an unavoidable association with oral microflora. This study is comparable to the current study done which inferred that the time

Table 1: Distribution of microorganisms isolated from individuals at 1 day usage of removable dental prosthesis

Microorganism	*n*	%
Streptococcus species	15	50
CONS	2	6.7
Staphylococcus aureus	17	56.7
Candida albicans	19	63.3
Klebsiella pneumoniae	3	10

Table 1 and Figure 3 show the percentage distribution of microbial species isolated from number of individuals on the first day of denture insertion. Microorganisms such as Streptococcus species, Coagulase-negative *staphylococcus, Staphylococcus aureus, Candida albicans* and *Klebsiella pneumoniae* were found in certain percentage. Among the isolated microorganism, *Candida albicans* showed the highest percentage which was found to be 63.3% with *n*=19. *Staphylococcus aureus* was found to be 56.7% with *n*=17. *Streptococcus species* was found to be 50% with *n*=15. The presence of *Diphtheroid, E. coli, Micrococcus species, Lactobacillus species, Enterococcus and pseudomonas* was not found in this group

Table 2: Distribution of microorganisms isolated from individuals at 1-month usage of removable dental prosthesis

Microorganisms	*n*	%
Streptococcus species	21	70
Coagulase-negative staphylococcus (CONS)	7	23.3
Staphylococcus aureus	23	76.7
Candida albicans	21	70
Klebsiella pneumoniae	5	16.7
Diptheroid	7	23.3
E. coli	4	13.3
Micrococcus species	1	3.3
Lactobacillus species	3	10
Enterococcus species	7	23.3
Pseudomonas species	1	3.3

Table 2 and Figure 4 show the percentage division of microbial variety secluded from number of persons at first month practice of removable dental prosthesis. It showed an increase in all the microorganisms among which the highest percentage of *Staphylococcus aureus* was found which was 76.7% with *n*=23. *Candida albicans* was found to be 70% with *n*=21. A few microorganisms which were not found at the time of denture insertion such as *Diphtheroid, E. coli, Micrococcus species, Lactobacillus species, Enterococcus and Pseudomonas* were also evident. The least percentage distribution was found to be 3.3% of *Micrococcus species* and *Pseudomonas* with *n*=1

of usage of removable prosthesis demonstrated a significant growth in the microorganisms.

Microflora ecosystem in oral cavity is essential to maintain oral and systemic balance. Any disturbance in the equilibrium of microflora of the oral cavity encouraged the growth of the pathogenic microorganism leading to oral diseases. Since the oral cavity acts as the primary gateway to the body, pathogenic microorganism from oral cavity gets smooth access to other body parts, causing systemic diseases. Both dental and medical practitioners are required to be acquainted with the effects of normal oral microflora; therefore, their treatment planning should target the control rather than suppression of oral microflora. Maintenance of good oral hygiene plays a key role in keeping our bodies healthy and preventing spread of infection to other body parts.

Denture-wearing patients are at high risk to develop denture stomatitis associated with Candida (CADS) because of the transformation of normal commensal Candida species into a pathogen in favourable conditions. Conditions resulting in oral imbalance, causing an unhealthy and unsuitable environment for denture wearing are immunocompromised condition, trauma caused by prosthesis or some systemic conditions and inadequate denture maintenance. Around 65–70% of denture wearers worldwide have been identified with occurrences of denture stomatitis caused by Candida. In a survey done by Vinaya Bhat V *et al*.[8] in 2013, it was stated that denture stomatitis with candida association prevails in denture wearers. Denture wearing men were more affected by Candida associated denture stomatitis than women making *Candida albicans* most prevalent in causing denture stomatitis, followed by *C. tropicalis* and C. glabrata

Figure 2: Blood agar culture plate growing the most commonly found bacteria in dental prosthesis

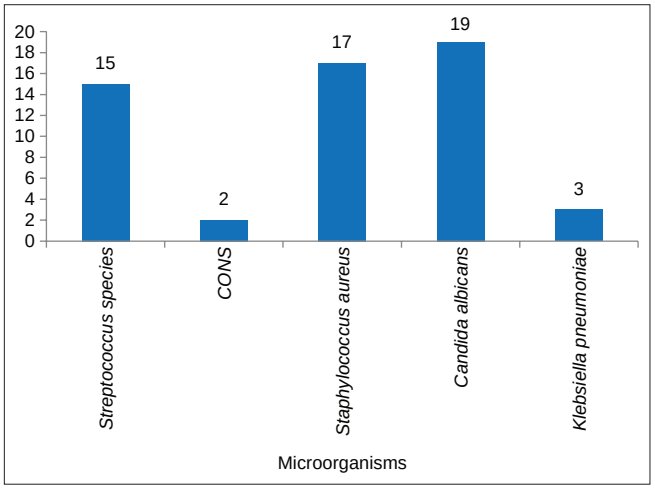

Figure 3: Statistical evaluation and comparison of different microorganisms at the day of denture insertion

Taebunpakul P *et al*.[9] studied the presence of *Candida* on palatal and denture surface and investigated the factors associated with denture stomatitis. Denture stomatitis is a common inflammatory reaction in denture-wearing patients. It was believed that palatal inflammation in denture stomatitis to be associated with *Candida* colonization. Denture-wearing patients were evaluated for denture stomatitis based on Newton's classification. Samples were collected from palatal surface and denture surface for Candida culture. Other predisposing factors having association with denture stomatitis were also evaluated by a questionnaire and prostheses evaluation. There was no association found between the amount of Candida and Denture stomatitis.

Pereira GA *et al*.[10] isolated, quantified, identified and compared opportunistic microorganisms of prostheses fitting surfaces, the hard palate and mouth rinses of individuals wearing removable maxillary prosthesis with (50 samples) and without (50 samples) lesions of denture stomatitis (DS). The strains were collected and identified using phenotypic, biochemical and molecular tests. The distribution of microorganisms was found to be significantly higher ($p < 0.05$) in the group of individuals with DS. *Candida albicans* was the primarily isolated yeast species in both groups followed by *C. tropicalis and C. glabrata.* Six other isolates identified were *C. dubliniensis, S. aureus* and *S. epidermidis* as most frequent *Staphylococcus species* in both groups [Table 4 and Figure 6]. *Klebsiella pneumoniae* was the major species in both groups [Table 5 and Figure 7]. The association between *Candida* species and bacteria isolated in this study with DS suggested that these microorganisms played an important role in the development of the disease [Table 6 and Figure 8].

Oral health care and prevention of oral disease is a primary requirement for quality life. It has been believed that aging cause progressive increase in microorganisms in the oral cavity. Lotfi-Kamran MH *et al*.[11] elaborated that the sole factor of denture wearing affected the number of *Candida species* detected as well as total *Candida* counts, uninfluenced by the age of

Table 3: Distribution of microorganisms isolated from individuals at 3-month usage of removable dental prosthesis

Microorganisms	*n*	%
Streptococcus species	23	76.7
CONS	13	43.3
Staphylococcus aureus	25	83.3
Candida albicans	24	80
Klebsiella pneumoniae	8	26.7
Diptheroid	17	56.7
E. coli	11	36.7
Micrococcus species	3	10
Lactobacillus species	9	30
Enterococcus species	13	43.3
Pseudomonas species	1	3.3

Table 3 and Figure 5 show the percentage distribution of microbial species isolated from number of individuals at the third month of usage of removable dental prosthesis. It showed that there was overall increase in all the microorganism which was found. It shows the highest percentage distribution of *Staphylococcus aureus* among all the isolated microorganisms which was found to be 83.3% with *n*=25. *Candida albicans* was found to be 80% with *n*=24. *Streptococcus species* was found to be 76.7% with *n*=23. The least percentage distribution was found to be 3.3% in *Pseudomonas* with *n*=1

Table 4: Distribution of gram-positive microorganisms isolated from individuals at 1 month usage of removable dental prosthesis

Microorganisms	*n*	%
Streptococcus species	21	70
CONS	7	23.3
Staphylococcus aureus	23	76.7
Diphtheroid	7	23.3
Micrococcus species	1	3.3
Lactobacillus species	3	10
Enterococcus species	7	23.3

Table 4 and Figure 6 show the distribution of gram-positive microorganisms isolated from individuals at 1-month usage of removable dental prosthesis that depicts the presence of the highest number of *Staphylococcus aureus* with *n*=23 followed by *Streptococcus species* with *n*=21

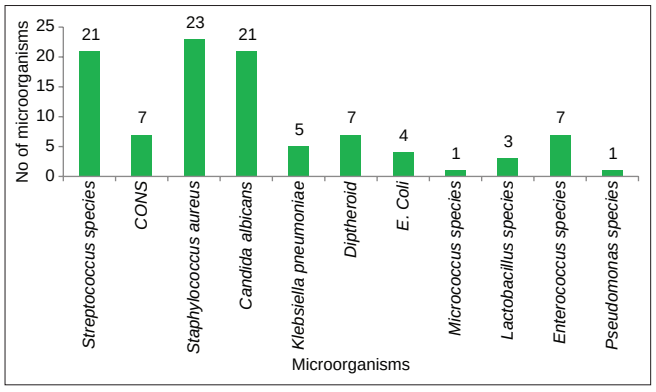

Figure 4: Statistical evaluation and comparison of different microorganisms at 1 month of usage of removable dental prosthesis

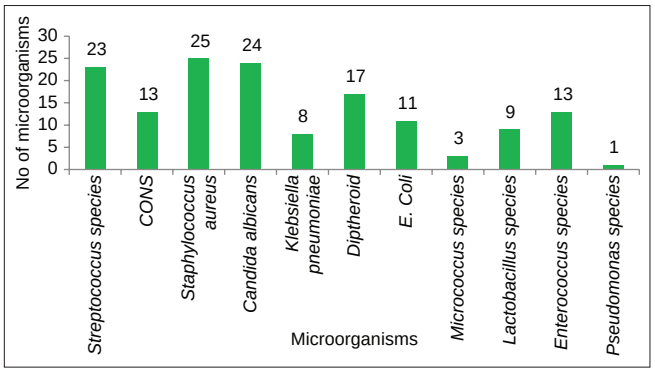

Figure 5: Statistical evaluation and comparison of different microorganisms among the three studied groups at third month of usage of removable dental prosthesis

the denture-wearing patient. Frequent incidents of multiple *Candida species* in denture-wearing patients distinguished them from patients who were not denture wearers. As a significant factor, denture wearing should not be overlooked while providing oral hygiene instructions for middle-aged patients [Table 7].

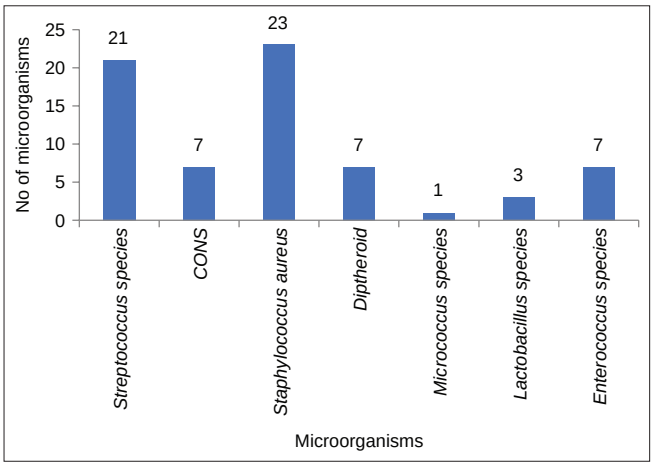

Figure 6: Statistical evaluation of gram-positive microorganisms isolated from individuals at 1-month usage of removable dental prosthesis

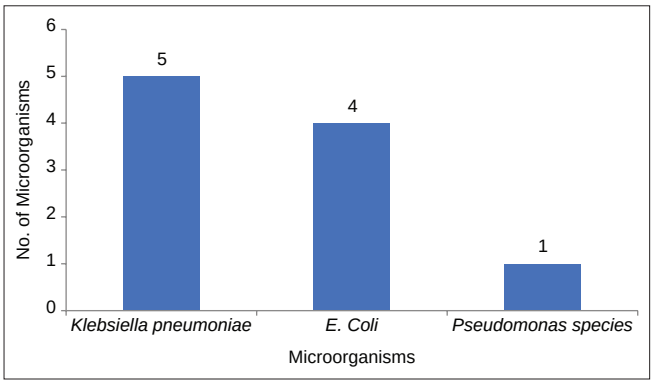

Figure 7: Statistical evaluation of gram-negative microorganisms isolated from individuals at 1-month usage of removable dental prosthesis

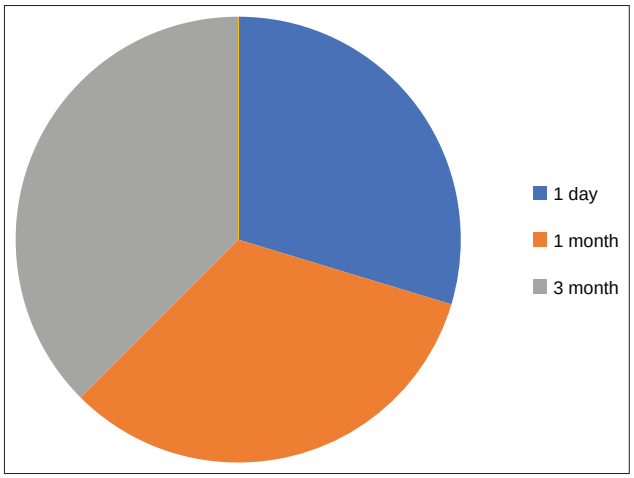

Figure 8: Statistical representation of *C. albicans* isolated from individuals using removable dental prosthesis at different intervals

The study thus demonstrated that the duration of usage of removable dental prosthesis significantly increased the microbial contamination and *Candida sp*. also [Tables 6,7 and Figure 8]. In denture wearers, these changes could persevere and would affect plaque formation with major pathogenic microorganisms. A progressive increase in microbial contamination was directly proportional to the duration of usage of removable prostheses. There was no significant difference in microbial colonization between partial and complete denture patients. The presence of all the identified microorganisms in high density was noted among individuals using dental prosthesis for more than 3 months in comparison to other groups.

Table 5: Distribution of gram-negative microorganisms isolated from individuals at 1-month usage of removable dental prosthesis

Microorganisms	*n*	%
Klebsiella pneumoniae	5	16.7
E. coli	4	13.3
Pseudomonas species	1	3.3

Table 5 and Figure 5 show the distribution of gram-negative microorganisms isolated from individuals at 1-month usage of removable dental prosthesis. It shows that the *Klebsiella pneumoniae* was found to be highest among gram-negative microorganism followed by *E. coli* and *Pseudomonas species*

Table 6: Distribution of microorganisms isolated from individuals using removable dental prosthesis at different intervals

Microorganisms	*n*		
	1	2	3
Streptococcus species	15	21	23
CONS	2	7	13
Staphylococcus aureus	17	23	25
Candida albicans	19	21	24
Klebsiella pneumoniae	3	5	8
Diphtheroid	0	7	17
E. coli	0	4	11
Micrococcus species	0	1	3
Lactobacillus species	0	3	9
Enterococcus species	0	7	13
Pseudomonas species	0	1	1

Table 7: Distribution of *Candida albicans* isolated from individuals using removable dental prosthesis at different intervals

	1 day	1 month	3 month
Candida albicans	19	21	24

Table 7 shows the distribution of *Candida albicans* isolated from individuals using removable dental prosthesis at different intervals. It shows that as the duration of usage increases, there is increase in the incidence of *Candida albicans*

Clinical significance

Oral microbes have been suspected in bacterial endocarditis, gastrointestinal infection and chronic obstructive pulmonary disease in general population among which the denture wearers offer a reservoir for microorganisms associated with these infections. Healthy individuals wearing removable prostheses must also be screened for potential sources of pathogenic microorganisms. In elderly patients, controlling microbial contamination through appropriate denture management guidance is crucial for promoting general health.

CONCLUSION

It is essential to investigate and evaluate microorganism contamination of dental prostheses to sustain a healthy oral function. Individuals with well-fit dentures must also be well thought out as potential sources of pathogenic microorganisms. Decontamination of removable dental prosthesis as in regular denture maintenance should be done to avoid and manage microbial contamination. Effective denture hygiene and cleansing to control denture microbial biofilm and overcome associated oral and systemic diseases is suggested. The prostheses at all times should be stored and maintained in a disinfected environment. It was suggested that patients should be well-informed with regard to prostheses cleanliness and usual follow-ups.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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