

## Temporal Alterations in Salivary Microflora Following Complete Removable Denture Insertion: A Longitudinal In Vivo Study

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### **Abstract:**

Placement of complete removable dentures (CRDs) in edentulous subjects causes a radical alteration of the oral environment, modifying salivary composition and increasing microbial colonization. The denture base, composed primarily of Polymethyl Methacrylate (PMMA), is an unshedding surface, facilitates biofilm formation. In the long term, this may cause change in oral microbial flora, predisposing the subject to infection such as denture stomatitis and mucosal infections. While widespread use of complete removable dentures (CRDs) has occurred, limited longitudinal data exist regarding the change in microbial dynamics over time and their association with clinical variables.

The present longitudinal in vivo study was conducted to assess modification of microbial salivary flora in 35 edentulous subjects at three successive intervals: prior to denture placement (T0), one month after placement (T1), and three months after placement (T2). Unstimulated saliva was collected and plated on selective media to provide colony-forming units (CFUs) of *Streptococcus pyogenes*, *Streptococcus mutans*, *Candida albicans*, *Escherichia coli*, and *Bacteroides fragilis*. Concurrent clinical assessment of oral hygiene, mucosal status, and denture fit were taken. Statistical comparison

revealed *Candida* spp. exhibited linear increase with maximal counts at T2, which correlated significantly with mucosal lesions ( $r = 0.90$ ,  $p < 0.001$ ). *Streptococcus pyogenes* and *S. mutans* were highest at T1 and reduced at T2, while *E. coli* and *B. fragilis* increased steadily.

The results indicate that time-dependent microbiological changes following complete removable denture placement are best related to prosthetic hygiene status and fit. An active approach through patient education, microbial screening on a routine basis, and early clinical intervention can minimize the risk of infection with the prosthesis and ensure improved patient outcomes and success of the denture in the long term.

**Keywords:** Complete removable dentures; Salivary microflora; *Candida albicans*; *Streptococcus mutans*; Denture stomatitis; Oral hygiene.

## 1. Introduction

### 1.1 Background and Clinical Relevance

Tooth loss, especially in elderly people, is a key issue in dental public health because it affects mastication, phonetics, facial appearance, and general well-being.<sup>1</sup> Complete removable dentures (CRDs) have been a useful prosthodontic rehabilitation for edentulous patients over the past decades that provides functional and aesthetic restoration.<sup>2</sup> Yet, while utilized very extensively and being inexpensive, complete removable dentures (CRDs) add extraneous material into the oral cavity and thus create a prosthetic environment that stimulates microbial colonizing as well as biofilm formation.<sup>3</sup> The oral cavity contains far more than 700 bacteria, fungal, viral, and archaeal species that form a dynamic multicomponent ecological system called the oral microbiome.<sup>4</sup> This balanced human oral microbiome exists symbiotically with the host and serves important roles in nutrition metabolism, immunity, and mucosal defense.<sup>5</sup> Stability of the oral environment is, however, tenuous and easily disrupted by external conditions including the administration of antibiotics, immunosuppression, and wear of removable prosthetic appliances and results in oral dysbiosis.<sup>6</sup>

Complete dentures, which consist mainly of Polymethyl Methacrylate (PMMA), have physical characteristics that facilitate the colonization of microorganisms due to their surface-porosity and water-retentive properties.<sup>7</sup> This enables numerous different pathogens to settle on the denture base and establish stable polymicrobial biofilms, particularly in regions with poor mechanical self-cleansing such as the palatal mucosa.<sup>8</sup> Biofilms that form are more tolerant to chemical disinfectants and contain immune responses after establishment.<sup>9</sup> Thus, complete removable dentures (CRDs) can also serve as long-term reservoirs for pathogens like *Candida albicans*, *Streptococcus mutans*, *Lactobacillus*, and anaerobic bacteria like *Bacteroides fragilis*.<sup>10,11</sup>

### 1.2 Oral Microbial Shifts and Denture Surfaces

Insertion of complete denture has a profound effect on oral microbalance.<sup>12</sup> Evidence has ever shown that the surface of acrylic dentures offers optimal support to microbial adhesion and biofilm formation.<sup>13</sup> These biofilms are initially composed of early colonizers like *Streptococcus* species but become multi-bacterial complex communities with anaerobic bacteria and fungi with aging.<sup>9</sup> Salivary pellicle formation, a protein film on denture surfaces increases microbial attachment<sup>14</sup>, and supports ecological succession.

The most significant among them is pathogenic yeast *Candida albicans*, commensal amongst all but opportunistic when favorable conditions exist.<sup>3</sup> It adheres strictly to PMMA surfaces and may also develop biofilms alone or synergistically with bacteria like *Streptococcus mutans*, thereby becoming even more virulent and resilient.<sup>9</sup> It has been observed that 65% to 70% of denture wearers are likely to develop *Candida* colonization, commonly occurring in the palatal area beneath the denture base.<sup>15</sup>

Increased colonization by *Candida* and *Streptococcus mutans* is contributed to by inadequate denture hygiene, extended denture use (overnight use being included too), and decreased salivary secretion.<sup>16</sup> Ill-fitting and rough denture bases also create pathogenic flora-promoting microenvironments and enhance mucosal inflammation.<sup>17</sup> Mechanical and microbiological factors thus synergize in the promotion of denture-associated infection, i.e., denture stomatitis.<sup>3,18</sup>

### 1.3 The Function of Saliva in Oral Microbial Homeostasis

Saliva is important for oral homeostasis by mechanical cleansing, pH buffering, and the secretion of antimicrobial peptides like lysozyme, lactoferrin, and secretory immunoglobulin A.<sup>19</sup> In the geriatric patients, though, the rate of salivary flow will be reduced by physiological aging, polypharmacy thus weakening the natural defense system of the oral cavity.<sup>20</sup> Yurdukuru et al. (2001)<sup>21</sup> also observed denture placement to be responsible for enhanced salivary flow in the beginning, but subsequently it becomes regular or declines.

Decrease in saliva quantity and quality results in decreased acid environment that favors acidogenic and aciduric bacteria such as *Lactobacillus* and *Streptococcus mutans*, resulting in mucosal damage and tissue irritation.<sup>6</sup> The diminished antimicrobial action of saliva also facilitates fungal development such as *Candida albicans*, resulting in increased colonization and inflammation rates.<sup>3</sup> The compromised clearance of microbes and prosthetic covering of oral mucosa also hinder saliva from accessing certain mucosal sites, thereby promoting localized microbial proliferation.<sup>8</sup>

Denture stomatitis and *Candida* load have also been linked in some research to low pH and buffering capacity of saliva.<sup>22,23</sup> These studies indicate that salivary factor measurement and microbial counts after denture insertion can be predictors of risk for infection at the onset.<sup>24</sup> Saliva should never be considered a digestive fluid but more an oral microbial balance controller, particularly in denture wearers.

### 1.4 Denture Stomatitis and Systemic Effects

The most frequent inflammatory disorder in denture wearers is denture stomatitis, and it may be present in up to 65% of patients based on age, oral status, and systemic status.<sup>3</sup> Denture stomatitis clinically manifests as erythema, edema, and petechiae localized to the palatal mucosa, generally non-symptomatic but sometimes burning sensation or discomfort may be present.<sup>25</sup> Newton's categorization demonstrates. Denture stomatitis in three forms: localized inflammation (Type I), generalized erythema (Type II), and papillary hyperplasia (Type III).<sup>26</sup>

The main etiologic factor is *Candida albicans*, bacterial co-infection and ill-fit of the prosthesis which also cause mechanical trauma.<sup>13</sup> The high microbial loads under the denture base have also been ascribed to increased systemic inflammatory markers, particularly in immunocompromised patients / uncontrolled diabetes.<sup>27</sup> The oral cavity is a reservoir for systemic pathogens and denture biofilms have been causing pneumonia, infective endocarditis, and systemic infections.<sup>28</sup>

Further evidence indicates that microbiota colonizing denture surfaces exhibit systemic health impacts.<sup>18</sup> For instance, aspiration pneumonia has a direct link to colonizing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* on dentures among elderly residents in nursing homes. The results highlight the significance of an integrated understanding of denture-associated microbial alterations and their impacts beyond the oral cavity.

### 1.5 Recent Research Gaps

Even with all the research that has been done on microbial transformation in dentures, there are still many areas of uncertainty. Many studies are cross-sectional and provide a static rather than a dynamic view of microbial succession.<sup>10</sup> They might be based on self-reported hygiene data or visual examination as opposed to quantitative data such as colony-forming units (CFUs) or microbial sequencing. Others target *Candida* specifically and overlook bacterial synergists or overlook microbial data in conjunction with clinical parameters like denture adaptation and mucosal status.<sup>29</sup>

While molecular tools such as real-time PCR and 16S rRNA sequencing are of high-resolution microbiology, they are costly and not for routine dental practice in most clinics.<sup>30</sup> Therefore, realistic long-term research that utilizes culture-based tools to monitor microbial change over time and correlate them with disease outcomes.<sup>15</sup>

Apart from this, no investigation has followed multiple microbial species Simultaneously—*Streptococcus*, *Lactobacillus*, *E. coli*, and *Bacteroides*—and clinical parameters like oral wellness, denture accommodation, and lesions are yet to be studied.<sup>31</sup> The holistic strategy is important for the specific design of hygiene regimens, prevention in the form of antifungal activity, and prosthetic accommodation that would minimize microbial colonization and inflammation.

### 1.6 Study Rationale and Objectives

With the above constraints, the present study was conducted to assess changes in saliva microflora after placing complete removable denture for 3 months and analyze such changes with clinical measures such as denture retention, oral health status, and mucosal lesions. Microbial species used in this context—*Streptococcus* spp., *Streptococcus mutans*, *Candida albicans*, *Escherichia coli*, and *Bacteroides fragilis*—were chosen due to their clinical significance in prosthesis infections.<sup>11,15</sup>

Through three key sampling times of unstimulated saliva (pre-placement, one month, and three months), this study offers a longitudinal picture of microbial adjustment to prosthesis wear. Quantitation is easy to access in the clinical setting with selective media for microbial isolation. Lastly, agreement of microbial trends with clinical features observable to clinicians renders assessment of risk factors better and timing of preventive intervention more understandable.

Lastly, the common goal is to educate prosthodontists, hygienists, and general practitioners on major windows of microbial colonization and inflammation and thereby facilitate evidence-based post placement care, antifungal treatment, and hygiene training. This study, in a wider context, suggests the need for the incorporation of microbiological insight into prosthodontic planning to facilitate long-term prosthesis survival and patient health.

## 2. Methodology

This pilot longitudinal in vivo study was an attempt to examine the alteration in salivary microflora after the wear of complete removable dentures (CRDs). The study was undertaken in a natural dental practice setup at the Department of Prosthodontics, Crown and Bridge, and Oral Implantology, DJ College of Dental Sciences and Research. The main aim was to evaluate microbial dynamics of saliva in edentulous patients following CRD placement for three months. The secondary aim was to relate the microbial changes to clinical parameters like denture fit, oral hygiene, and oral lesion presence.

35 patients were taken for study sample by convenience sampling. Sample size was calculated based on expected microbial heterogeneity, and power considerations were met statistically.

Well-defined exclusion criteria were used to provide data uniformity and study population homogeneity. Only edentulous patients 50 years old and older, and first-time complete removable denture wearers, were included. The participants were asked to give signed written informed consent and stay at their current residential location for the entire study period. Additionally, participants who had not been given any antibiotics, corticosteroids, or immunosuppressants for the last three months were regarded as eligible to exclude confounding parameters from oral microbial flora.

Excluded were patients with systemic illness expected to upset oral microbial homeostasis, e.g., terminal illness, patients with cerebrovascular accidents, or psychiatric therapy patients. Excluded also were patients with alcoholic or drug-abuse history in the preceding year, or patients who had previously received microbial sampling or treatment of oral flora.

Saliva specimens were collected from all the subjects at three time-defined intervals to track temporal microbial variation: at baseline (T0) prior to denture insertion, at one month (T1) following denture insertion, and then at three months (T2). Unstimulated whole saliva was collected under baseline conditions, and at each step of collection and transportation, aseptic techniques were employed. Samples were as soon as possible shipped to the microbiology laboratory for culture and examination.

To separate and count changes in certain microbial species, differential and selective media were employed together. The alpha-hemolytic *Streptococcus* species were separated on blood agar. Sabouraud's Dextrose Agar (SDA) provided a growth and count medium for the *Candida* species. Mitis salivarius agar was utilized to separate *Streptococcus mutans* as it facilitates its selective growth. Besides that, Brain Heart Infusion (BHI) broth was employed to grow *Escherichia coli* and thioglycollate broth to grow anaerobes such as *Bacteroides fragilis*. The plates were incubated under ideal conditions and the colony-forming units (CFUs) of the saliva per microliter were counted with the help of a calibrated colony counter.

In addition to microbiological assessment, clinical parameters of oral hygiene status (based on visible plaque and mucosal status), adaptation of dentures (clinically assessed), and oral lesions (presence or absence of lesions, especially erythema

or pseudomembranous candidiasis) were also noted. This was done at a multivariate level so that measurement for microbial alterations versus mechanical or hygiene status of dentures could be correlated.

Data collected were statistically processed with the aid of the Statistical Package for the Social Sciences (SPSS). Means and standard deviations of CFU values for various time points were calculated. Repeated measures analysis of variance (ANOVA) was employed to determine significance of variation over time. Pearson correlation tests were utilized in the testing of correlation between microbial counts and clinical parameters such as denture fit, oral hygiene, and the presence or absence of stomatitis or other mucosal lesion. P-values < 0.05 were taken to be significant.

This well-designed and ethical study offers a solid foundation for the investigation of the effects of wear of complete dentures on oral microbiota and provides data relevant to the enhancement of prosthodontic treatment and preventive oral infection care.

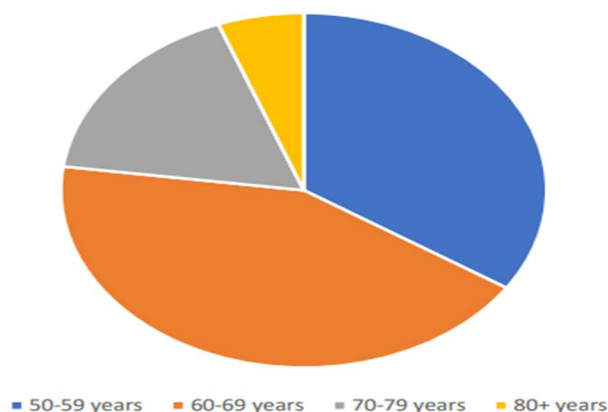
### 3. Results

#### 3.1 Demographic Characteristics of Study Population

**Table 1. Age Distribution of Study Population**

Age Group	Frequency	Percentage
50–59 years	12	34.3%
60–69 years	15	42.9%
70–79 years	6	17.1%
80+ years	2	5.7%
<b>Total</b>	<b>35</b>	<b>100%</b>

**Age Distribution of Study Population**

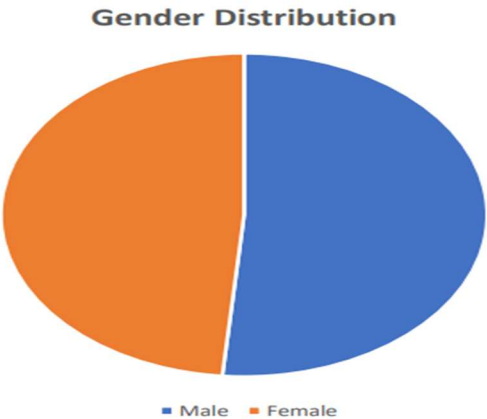


**Figure 1: Age Distribution of Study Population**

Table 1 presents the distribution of study participants across various age groups. Most subjects (42.9%) were in the 60–69 age range, followed by 34.3% in the 50–59 group. A smaller proportion belonged to the older age categories, including 70–79 years (17.1%) and above 80 years (5.7%). The skew towards the 50–69 age range reflects the typical demographic that seeks prosthodontic rehabilitation with complete dentures. Understanding age distribution is essential, as advancing age may influence oral microbial balance and immune responses, potentially altering the susceptibility to microbial colonization and denture-related infections.

**Table 2. Gender Distribution of Study Population**

Gender	Frequency	Percentage
Male	18	51.4%
Female	17	48.6%
Total	35	100%



**Figure 2: Gender Distribution of Study Population**

Table 2 details the gender composition of the sample, with 51.4% male and 48.6% female participants. This relatively even gender split allows for unbiased interpretation of microbial trends without a significant skew towards one sex. Gender-based physiological differences, such as salivary flow rates and hormonal influences on mucosal immunity, could subtly influence microbial dynamics; however, this balanced distribution minimizes such confounding effects in this study.

**3.2 Baseline Clinical Characteristics**

**Table 3. Oral Hygiene Status at Baseline (T0)**

Oral Hygiene Status	Frequency	Percentage
Poor	5	14.3%
Fair	15	42.9%
Good	15	42.9%
Total	35	100%

Oral Hygiene Status at Baseline

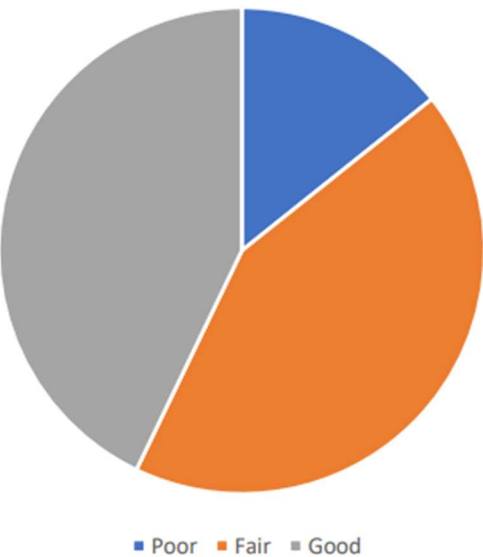
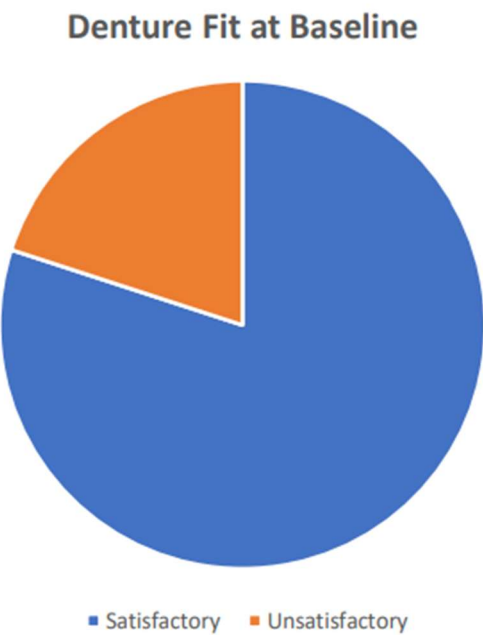


Figure 3: Oral Hygiene Status at Baseline (T0)

Table 3 summarizes the oral hygiene status of participants at baseline, before denture insertion. A majority of individuals were categorized as having fair (42.9%) or good (42.9%) hygiene, while only 14.3% were classified as having poor hygiene. These findings are relevant because baseline hygiene practices are known to influence the extent of biofilm development on prosthetic surfaces and mucosa. Individuals with poor hygiene at the outset are more likely to exhibit accelerated microbial colonization, particularly by opportunistic pathogens such as *Candida albicans*.

Table 4. Denture Fit at Baseline (T0)

Denture Fit	Frequency	Percentage
Satisfactory	28	80.0%
Unsatisfactory	7	20.0%
Total	35	100%



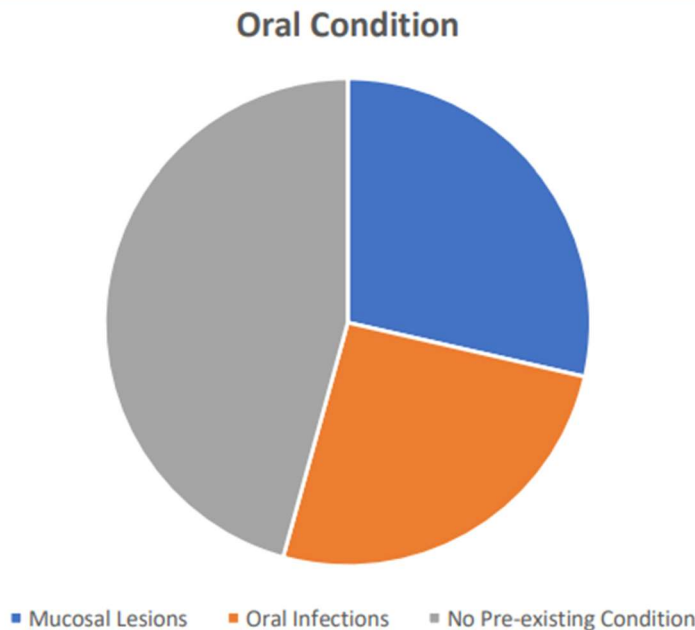
**Figure 4: Denture Status at Baseline (T0)**

This table assesses the clinical quality of denture fit at the point of insertion. A majority (80%) received dentures with satisfactory fit, whereas 20% had minor issues classified as unsatisfactory. Denture fit is a key mechanical factor influencing oral health in prosthetic wearers. Poorly fitting dentures can create trauma, encourage microbial retention under the denture base, and exacerbate mucosal lesions. The table lays the foundation for analyzing how denture adaptation may correlate with microbial changes over time.

**Table 5. Pre-existing Oral Conditions at Baseline (T0)**

Oral Condition	Frequency	Percentage
Mucosal Lesions	10	28.6%
Oral Infections	9	24.6%
No Pre-existing Issues	16	46.6%
Total	35	100%





**Figure 5: Pre-existing Oral Conditions at Baseline (T0)**

Table 5 documents the presence of mucosal lesions or infections before denture insertion. Notably, 28.6% had existing mucosal lesions, and 24.6% presented with oral infections, while 46.6% were free from any pathological conditions. These findings suggest that over half the sample had some level of compromised mucosal health at baseline, which may have predisposed them to faster or more severe colonization by oral pathogens. This information is vital in interpreting any correlation between baseline mucosal integrity and subsequent increases in microbial load.

**3.3 Microbial Composition Over Time**

**Table 6. Mean CFU Counts of Selected Microbial Species at Different Time Points**

Culture Medium	Time Point	Microbial Species	Mean CFU/μl
Blood Agar	T0	Streptococcus spp.	25.3
	T1	Streptococcus spp.	30.1
	T2	Streptococcus spp.	20.5
Sabouraud Dextrose Agar	T0	Candida spp.	15.7
	T1	Candida spp.	20.2
	T2	Candida spp.	25.9
Mitis Salivarius Agar	T0	Streptococcus mutans	10.6
	T1	Streptococcus mutans	12.4
	T2	Streptococcus mutans	8.8
Brain Heart Infusion Broth	T0	Escherichia coli	5.2

Thioglycollate Broth	T1	Escherichia coli	6.3
	T2	Escherichia coli	4.9
	T0	Bacteroides fragilis	8.1
	T1	Bacteroides fragilis	10.0
	T2	Bacteroides fragilis	12.6

Table 6 displays the average colony-forming unit (CFU) counts of five microbial species—*Streptococcus spp.*, *Candida spp.*, *Streptococcus mutans*, *Escherichia coli*, and *Bacteroides fragilis*—at three intervals: baseline (T0), one month (T1), and three months (T2) after denture insertion. The data illustrate dynamic changes in microbial colonization over time.

- *Streptococcus spp.* increased from T0 to T1, followed by a decrease at T2, suggesting initial colonization followed by partial re-equilibration.
- *Candida spp.* showed a steady and significant increase across all time points, highlighting its ability to persist and dominate in the altered denture environment.
- *Streptococcus mutans* peaked at T1 and declined slightly at T2, indicating transient dominance possibly related to biofilm maturation.
- *E. coli* and *B. fragilis* remained relatively low but showed a mild increasing trend, suggesting that anaerobic and enteric colonization may occur under the denture base over time.

3.4 Association Between Microbial Changes and Clinical Parameters

Table 7 presents Pearson correlation coefficients between microbial species and clinical parameters, including denture fit, oral hygiene, and presence of mucosal lesions. Strong positive correlations were observed between:

- *Candida albicans* and mucosal lesions (**r = 0.90**), confirming its key role in denture stomatitis.
- Poor denture fit and *Streptococcus mutans* growth, emphasizing the impact of prosthesis adaptation on bacterial colonization.
- Improved hygiene and reduced *Lactobacillus* levels (**negative correlation**), reinforcing the preventive role of effective denture hygiene in managing acidogenic bacteria.

These correlations offer crucial insights into how clinical conditions affect microbial load and composition, thereby validating the multifactorial etiology of prosthetic-related oral infections.

Table 7. Association Between Microbial Changes and Clinical Parameters

Clinical Parameter	Microbial Species	Time Point	Pearson's r	p-value
Denture Fit	Streptococcus mutans	T0	0.75	<0.05
		T1	0.68	
		T2	0.72	
Oral Hygiene Status	Lactobacillus spp.	T0	-0.82	<0.01
		T1	-0.75	
		T2	-0.80	
Presence of Lesions	Candida albicans	T0	0.90	<0.001

		T1	0.85	
		T2	0.88	

#### 4. Discussion

The study was conducted in the present investigation to assess temporal alterations in salivary microflora post placement of complete removable dentures (CRDs) and their relationship with significant clinical parameters like denture adaptation, oral hygiene status, and onset of oral lesions. The results indicate widespread and cumulative alterations in the oral microbiological milieu with signs of dynamic interaction between prosthetic surface and oral flora.

##### Microbial Patterns and Transitions Through Time

The most significant finding of this research was the evident trend of microbial change after denture placement. The research revealed that *Streptococcus* species, early prominent colonizers of the oral cavity, were boosted in the first first month (T1) after denture placement but at the third month (T2) were lowered. This first peak is caused by colonization of the new surfaces of the prosthesis at a high rate, mostly the acrylic base, which is a good substrate for plaque development. The dip that occurs afterward can be caused by stabilization of the microflora following better oral hygiene practices or host immune adaptation with time. This concurs with Marsh et al. (1992)<sup>12</sup> and Oizumi et al.'s (1994) research in that they also reported interim spikes of *Streptococcus* species during the adaptation stage. Conversely, *Candida* species showed consistent and considerable rise at each interval.

From a mean of 15.7/μl at T0 to 25.9/μl at T2, the trend shows prolonged colonization and establishment of fungal flora. It was found that Acrylic denture surfaces are prone to retain food particles and water, which is a state conducive to fungal growth. In addition, compromised salivary function among elderly individuals may also decrease antifungal protective defenses, and therefore predispose them to *Candida* hypergrowth. The findings of this research lean towards the positive that, consistent with the literature, use of dentures is linked with being highly colonized with *Candida albicans* and vulnerable to denture stomatitis (Gendreau & Loewy, 2011).<sup>3</sup> *Streptococcus mutans* counts were at their highest at T1 and fell at T2, consistent with the trend observed with *Streptococcus* spp. in total.

This difference is indicative of a need for their early observation of cariogenic species levels while wearing dentures for several months. Ill fitting dentures, plaque buildup, and compromised self-cleaning capability of oral tissues can potentially lead to *S. mutans* overgrowth. As expected, the research showed that denture wearers with a poor denture fit had a positive correlation with high *S. mutans* counts, as consistent with findings by Mihalow & Tinanoff (1988) between prosthetic defect detection and cariogenic biofilm growth. Presence of Anaerobic and Opportunistic Species

**Notwithstanding the low *Escherichia coli* counts and *Bacteroides fragilis* levels throughout the experiment, their occurrence is of clinical significance.**

These bacteria, which are not normally present in the oral microbiome, suggest possible contamination with outside sources or disturbed local oral conditions secondary to prosthesis. The increasing trend of *Bacteroides fragilis* from 8.1 to 12.6 CFU/μl over three months may suggest the development of anaerobic microecologies between the denture base, particularly in cases of poor maintenance. This observation substantiates previous microbiological findings that had demonstrated anaerobic niches among denture wearers (Sato et al., 1993).

**Association with Clinical Parameters**  
The high correlation ( $r = 0.90$ ,  $p < 0.001$ ) between *Candida albicans* counts and oral lesions unequivocally indicates that high fungal load is a significant etiologic factor in denture stomatitis. This would imply clinically that monitoring and early treatment with antifungal medication could be justified in progressive *Candida* increases. The inverse correlation between enhanced oral hygiene and *Lactobacillus* species also indicates patient education in acidogenic flora management and oral microbial homeostasis. The fact that the proper fit of dentures in participants had fewer *Streptococcus mutans* levels and fewer mucosal lesions of oral mucosa is also worthy of mention. This also highlights double significance of mechanical and behavioral factors for the control of microbial colonization. Dysfunctional dentures not only compromise the mucosa but also provide a site of retention for biofilm, as discussed in Zlatarić et al.

(2002)<sup>17</sup> and Redfern et al. (2022).<sup>18</sup>

### Comparative Context and Broader Implications

The results of this study is in co-ordination with the rest of prosthetic-induced dysbiosis literature.

A number of research studies have established that introducing a foreign body, in this case an acrylic denture, upsets the balance of the oral ecosystem. These alterations do not happen on prosthetic surfaces exclusively but on surrounding mucosal tissues and even on the overall status of immunocompromised subjects. Veseli et al. (2023) and Ribeiro et al. (2022)<sup>27</sup> through a study also highlight the need for denture cleanliness in impacting not just oral inflammation, but also cardiovascular and systemic parameters, validating the need for an interdisciplinarity approach in prosthodontics. Preventively, stepwise accumulation of fungal burden over time would imply that early antifungal prophylaxis, particularly in high-risk patients (i.e., diabetic or immunocompromised patients), would be considered. Future research with the use of metagenomic or 16S rRNA sequencing might provide additional information on microbial change and resistance patterns, as illustrated in the example of Yacob et al. (2024).<sup>31</sup>

### 5. Conclusion

This study brings out the fact that full wearing of removable dentures induces consistent and cumulative alterations in the fully edentulous patients' salivary microflora. The most noticeable alteration was a steady increase in *Candida albicans* counts over the course of the three months, which had a high correlation with mucosal lesions as well as clinical manifestations of denture stomatitis. A temporary spike in *Streptococcus* spp. was also seen. and *Streptococcus mutans* one month following insertion. This suggests that initial microbial colonization is an active process to be closely monitored. Trends here suggest that the oral environment passes through a period of microbial instability following insertion of the dentures where opportunistic microorganisms have the potential to assume predominance.

Mechanical and behavioral influences were observed to have effects on such microbial changes. Poor-fitting dentures had caused local trauma and retention areas for biofilm, and poor oral hygiene invited colonization with acidogenic and pathogenic bacteria. *Escherichia coli* and *Bacteroides fragilis*, the opportunistic pathogens but in lower numbers, exhibited gradual but predictable rise, indicating the likelihood of more complex microbial colonization in the longer term. These findings suggest the importance of addressing prosthesis design, for effective fit adequacy, and for effective reinforcement of hygiene from the initial stages of denture wear. Patient education tailored to the individual and periodic clinical follow-up could noticeably reduce the risk of infection and improve long-term success of prosthetic rehabilitation.

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