

Correlation between Bacterial Load of Streptococcus Mutans and Lactobacillus with Gluten-rich or gluten-free diet in patients with celiac disease

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Abstract

Introduction: Celiac disease is a multifactorial autoimmune gastrointestinal disorder. The aim of this study was to investigate the association between the frequency of salivary *Streptococcus Mutans* and *Lactobacillus* bacteria with gluten-containing and gluten-free diets in patients with celiac disease.

Materials and Methods: The present study was a descriptive-analytical cross-sectional research conducted in 2025 on 75 participants, including 25 patients with celiac disease following a gluten-free diet, 25 patients with celiac disease consuming a gluten-containing diet, and 25 healthy individuals as the control group. Sampling was performed using a convenience method among patients with celiac disease. Demographic information and oral health indices were collected using a researcher-made questionnaire. Saliva samples were collected using a non-stimulated method and analyzed to determine the colony counts of *Streptococcus mutans* and *Lactobacillus acidophilus*. Dental caries were assessed using the DMFT index. Data analysis was performed using SPSS software version 26. Descriptive and inferential statistical tests, Pearson correlation test, Spearman test, independent t-test, and chi-square test were applied for data analysis.

Results: The mean age of participants in the control group, gluten-free diet group, and gluten-containing diet group was reported 29.8 ± 4.9 and 27.16 ± 5.13 , and 29.24 ± 4.0 years, respectively. Comparison of *Streptococcus mutans* load among the study groups showed a statistically significant difference in colony counts between the three groups ($p < 0.001$). The number of *Lactobacillus acidophilus* colonies also demonstrated a statistically significant difference among the three groups ($p < 0.001$). The findings indicated that the mean DMFT index in patients with celiac disease consuming a gluten-containing diet was significantly higher than that of patients adhering to a gluten-free diet ($p < 0.001$).

Conclusion: A gluten-containing diet in patients with celiac disease is associated with an increased load of cariogenic bacteria in saliva. These findings emphasize the importance of adherence to a gluten-free diet not only in reducing gastrointestinal symptoms but also in decreasing the risk of dental caries in patients with celiac disease.

Introduction

Celiac disease is a multifactorial autoimmune disorder that is associated with damage to the small intestine and is triggered by the consumption of gluten-containing foods, wheat, and related prolamins found in barley and rye in genetically predisposed individuals (1). Most individuals affected by celiac disease experience gastrointestinal symptoms such as abdominal pain, bloating, vomiting, and weight loss; however, in some patients, other manifestations such as anemia, headache, skin rashes, and joint pain may also be identified (2). The prevalence of celiac disease in the general population worldwide is approximately 1% (3), and it has been reported to be more common in women than in men. This difference may be attributed to hormonal changes as well as genetic factors; moreover, women tend to seek medical care more frequently than men (4). The prevalence of celiac disease is higher among

individuals with a history of other autoimmune disorders, including type 1 diabetes mellitus, for which the prevalence has been reported to range between 1.6% and 16/4% (4).

The most commonly reported symptoms include vomiting, abdominal pain, weight loss, and malabsorption (5). Diagnosis of celiac disease is based on positive serology for anti-tissue transglutaminase IgA (Anti-TTG-IgA), anti-endomysia antibodies, and villous atrophy observed in small intestinal biopsy specimens (5). In many diagnostic protocols, Anti-TTG IgA levels exceeding ten times the upper limit of normal are considered a strong criterion for the diagnosis of celiac disease in both symptomatic adults and children (6). The treatment of celiac disease consists of a lifelong gluten-free diet (GFD) (7).

Since the oral cavity represents the first segment of the gastrointestinal tract, it can also be considered one of the sites for the manifestation of celiac disease (8). Common oral manifestations of celiac disease include recurrent aphthous stomatitis, dental enamel defects, delayed tooth eruption, multiple dental caries, angular cheilitis, and atrophy of lingual papillae, xerostomia, and burning sensation of the tongue (9).

Some researchers believe that clinical manifestations such as early-onset dental caries without an identifiable cause may serve as indicators of underlying systemic diseases, including celiac disease (10). Furthermore, dental caries influenced by *Streptococcus mutans* and *Lactobacillus acidophilus* may represent systemic manifestations of celiac disease (5, 11). Scientific evidence has also demonstrated that the bacterial load of *Streptococcus mutans* and *Lactobacillus acidophilus* in the saliva of patients with celiac disease is significantly affected by the type of diet (12). Therefore, an increased load of these bacteria recognized as the primary etiological agents of dental caries may provide a plausible explanation for the higher prevalence of caries and increased DMFT index observed in patients with celiac disease who consume gluten (13).

Studies have shown that the DMFT index is higher in children with celiac disease (14). Other findings indicate that among individuals seeking treatment for dental caries, the prevalence of positive celiac serological tests is higher than in the general population. These results suggest that celiac disease may remain undiagnosed in some individuals with dental caries and may present primarily through non-gastrointestinal symptoms (15).

Simultaneous consideration of dietary therapy, salivary microbial status, and oral health indices including dental caries, salivary pH, and bacterial load is of significant importance in patients with celiac disease. As a major health concern in this patient population, such an approach may facilitate the development of targeted preventive programs aimed at maintaining oral and dental health alongside management of the underlying disease. Therefore, the present study was conducted to investigate the association between the frequency of salivary *Streptococcus mutans* and *Lactobacillus* bacteria with gluten-containing and gluten-free diets in patients with celiac disease.

Materials and Methods

The present study was a descriptive–analytical cross-sectional study conducted in 2025. The study population included patients with celiac disease who were either following a gluten-free diet or consuming a gluten-containing diet. The sample size was calculated based on the findings of the study by Acar et al. (5), considering the salivary *Streptococcus mutans* load in patients with celiac disease (48.65%) and in the control group (14%). Accordingly, 25 participants were allocated to each group, resulting in a total sample size of 75 patients.

p1	0.48	$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 (p_1 q_1 + p_2 q_2)}{(p_1 - p_2)^2}$
p2	0.14	
a	0.05	
b	0.2	

Convenience method among patients diagnosed with celiac disease. Inclusion criteria consisted of patients aged 20 to 40 years with a confirmed diagnosis of celiac disease based on endoscopic evaluation, intestinal biopsy, and serological tests, as well as completion of a written informed consent form for participation in the study. Exclusion criteria included the presence of other gastrointestinal diseases or psychiatric disorders, a history of antibiotic consumption within the past two weeks, use of proton pump inhibitors, consumption of probiotics, smoking or alcohol use, and undergoing dental treatments within the previous three months. The data collection tool is a researcher-made questionnaire that includes a checklist of demographic information and data related to systemic diseases (age, sex, medical history, medication use, etc.). In addition, a specific questionnaire was used for saliva collection to assess the frequency of *Streptococcus mutans* and *Lactobacillus* bacteria and to measure salivary pH. The present study was approved by the Ethics Committee of Qom University of Medical Sciences under the code IR.MUQ.REC.1404.088. Subsequently, sampling was conducted among 25 patients with celiac disease adhering to a gluten-free diet, 25 patients with celiac disease consuming a gluten-containing diet, and 25 healthy individuals. A minimum disease duration of two years since diagnosis was considered. Classification was performed according to the approved Marsh Classification system, which categorizes the progression from normal intestinal mucosa to flattened mucosa in celiac disease (16).

Non-stimulated saliva samples were collected between 9:00 and 11:00 a.m. Approximately 2 mL of saliva was collected from each participant into sterile microtubes. Participants were instructed to refrain from eating, tooth brushing, and salivary stimulation for at least 30 minutes prior to sampling (17, 18).

To measure *Streptococcus mutans*, diluted saliva samples were transferred into BHI Broth (selective liquid medium) using microtubes and incubated at approximately 37°C for 24 to 72 hours until turbidity appeared as an indicator of bacterial growth. Following turbidity, streak culture was performed on Blood Agar plates, which were then placed in a standard incubator. After incubation, plates were examined for colony formation, and colonies consistent with the morphological characteristics of *Streptococcus mutans* were selected. Colony-forming units were counted based on sample dilutions and recorded as

CFU/ml. The BHI Broth and Blood Agar media used in this study were obtained from Merck, Germany (Figs. 1 and 2) (7).

For evaluation of *Lactobacillus acidophilus*, diluted saliva samples were transferred into MRS Broth (liquid medium for primary growth enhancement) using microtubes and incubated at approximately 37°C for 24 to 72 hours until turbidity appeared. Subsequently, streak culture was performed on MRS Agar using sterile swabs. Plates were placed in anaerobic jars containing a candle to create reduced oxygen conditions. After incubation, colonies with morphological characteristics consistent with *Lactobacillus acidophilus* were counted and recorded as CFU/ml. The MRS Broth and MRS Agar media used in this study were supplied by Liofilchem, Italy (Figs. 3 and 4) (19).

Salivary pH was measured using an AZ pH/mV Meter 8601 (AZ Instrument Corp., Taiwan). Each sample was measured twice, and the mean value was recorded as the final pH. To ensure accuracy, three-point calibration was performed using standard buffer solutions (pH = 2, pH = 7, and pH = 10) prior to sample measurements (17, 20).

The DMFT index was calculated based on the number of decayed, missing, and filled teeth due to caries. According to standard criteria, a DMFT index of 0–1 indicates normal status, 1–3 indicates low caries, 3–5 moderate caries, 5–7 high caries, and values more than 7 indicate severe dental caries (21). Statistical analysis was conducted using SPSS software version 26. Microbial colony counts were reported as $M \pm SD$. Descriptive and inferential statistical tests were used to present M, SD, frequencies, and percentages. Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. Independent t-test and Chi-Square test were used for analysis of relationships and differences. Comparison of colony counts among the three groups was performed using One-way analysis of variance (ANOVA) or the Kruskal–Wallis test, as appropriate. A significance level of less than 0.05% was considered statistically significant.

Results

The present study was conducted to evaluate the frequency of *Streptococcus mutans* and *Lactobacillus acidophilus* species in the saliva of patients with celiac disease compared with healthy controls in the city of Qom. The mean age of participants in the case and control groups was 29.8 ± 4.9 and 29.24 ± 4.0 years, respectively. The findings of the one-way analysis of variance (ANOVA) test demonstrated a statistically significant difference in the number of *Streptococcus mutans* colonies among the three studied groups ($p < 0.001$) (Table 1). Post-hoc Tukey analysis for multiple comparisons of *Streptococcus mutans* bacterial load revealed that the microbial load in patients with celiac disease consuming a gluten-containing diet was significantly higher than that of patients with celiac disease adhering to a gluten-free diet ($p < 0.001$). In addition, the bacterial load in both celiac groups was significantly higher compared with the control group ($p < 0.001$) (Table 2).

Table 1

Comparison of Streptococcus mutans microbial load in patients with celiac disease consuming gluten-containing and gluten-free diets compared with the control

Variable	Control group (N = 25) Mean $\pm$ S	Patients with celiac disease on a gluten-containing diet (N = 25) Mean $\pm$ S	Patients with celiac disease on a gluten-free diet (N = 25) Mean $\pm$ S	P.Value One-way Analysis of Variance (ANOVA)
Streptococcus Mutans microbial load	51.32 $\pm$ 10.5	130.68 $\pm$ 1.98	80.08 $\pm$ 15.92	0.001

Table 2

Multiple comparison of Streptococcus mutans bacterial load among study groups

Group	Subgroups	Mean of Difference	SE	P.Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Patients with celiac disease on a gluten-containing diet	Patients with celiac disease on a gluten-free diet	50.6	42.6	0.001	40.9	60.2
	Control group	79.3	42.6	0.001	69.6	89.4
Patients with celiac disease on a gluten-free diet	Patients with celiac disease on a gluten-containing diet	-50.6	42.6	0.001	-60.2	-40.9
	Control group	29.7	42.6	0.001	18.7	39.4
Control group	Patients with celiac disease on a gluten-containing diet	-79.3	42.6	0.001	-89.4	-69.6
	Patients with celiac disease on a gluten-free diet	-29.7	42.6	0.001	-39.4	-18.7

As presented in Table 3, the results of one-way ANOVA indicated a statistically significant difference in the number of *Lactobacillus acidophilus* colonies among the three groups

Table 3
Comparison of *Lactobacillus acidophilus* microbial load among study groups

Variable	Control group (N = 25) Mean $\pm$ S	Patients with celiac disease on a gluten-containing diet (N = 25) Mean $\pm$ S	Patients with celiac disease on a gluten-free diet (N = 25) Mean $\pm$ S	P.Value One-way Analysis of Variance (ANOVA)
Streptococcus mutans microbial load	40.84 $\pm$ 7.5	78.12 $\pm$ 14.18	71.28 $\pm$ 15.94	0.001

($p < 0.001$). Tukey post-hoc analysis showed no statistically significant difference in *Lactobacillus acidophilus* load between the two celiac groups; however, in both groups, the bacterial load was significantly higher compared with the control group ($p < 0.001$) (Table 4).

Table 4
Multiple comparison of *Lactobacillus acidophilus* bacterial load among study groups

Group	Subgroups	Mean of Difference	SE	P.Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Patients with celiac disease on a gluten-containing diet	Patients with celiac disease on a gluten-free diet	6.8	36.5	0.171	-21.9	15.7
	Control group	38.2	36.5	0.001	29.3	46.2
Patients with celiac disease on a gluten-free diet	Patients with celiac disease on a gluten-containing diet	-6.8	36.5	0.171	-15.7	21.9
	Control group	31.4	36.5	0.001	22.5	40.3
Control group	Patients with celiac disease on a gluten-containing diet	-38.2	36.5	0.001	-46.2	-29.3
	Patients with celiac disease on a gluten-free diet	-31.4	36.5	0.001	-40.3	-22.5

Analysis of the DMFT index demonstrated a statistically significant difference among the three study groups ($p < 0.001$) (Table 5). Furthermore, Tukey post-hoc test results indicated that the mean DMFT index in patients with celiac disease consuming a gluten-containing diet was significantly higher than that of patients following a gluten-free diet ($p < 0.001$). In addition, both celiac groups showed significantly higher DMFT values compared with the control group ($p < 0.001$) (Table 6).

Table 5
Comparison of DMFT index among study groups

Variable	Control group (N = 25) Mean ± SD	Patients with celiac disease on a gluten-containing diet (N = 25) Mean ± SD	Patients with celiac disease on a gluten-free diet (N = 25) Mean ± SD	P.Value One-way Analysis of Variance (ANOVA)
DMFT index	40.84 ± 7.5	8.08 ± 2.05	6.2 ± 1.26	0.001

Table 6
Multiple comparison of DMFT index among study groups

Group	Subgroups	Mean of Difference	S E	P.Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Patients with celiac disease on a gluten-containing diet	Patients with celiac disease on a gluten-free diet	1.8	0.42	0.001	0.86	2.7
	Control group	4.04	0.42	0.001	3.02	5.05
Patients with celiac disease on a gluten-free diet	Patients with celiac disease on a gluten-containing diet	-1.8	0.42	0.001	-2.7	-0.86
	Control group	2.1	0.42	0.001	1.1	3.1
Control group	Patients with celiac disease on a gluten-containing diet	-4.04	0.42	0.001	-5.05	-3.02
	Patients with celiac disease on a gluten-free diet	-2.16	0.42	0.001	-3.1	-1.1

The results of one-way ANOVA revealed that the mean salivary pH was higher in patients with celiac disease adhering to a gluten-free diet compared with the other two groups; however, no statistically significant difference was observed among the three groups (Table 7).

Table 7
Comparison of salivary pH among study groups

Variable	Control group (N = 25) Mean $\pm$ SD	Patients with celiac disease on a gluten-containing diet (N = 25) Mean $\pm$ SD	Patients with celiac disease on a gluten-free diet (N = 25) Mean $\pm$ SD	P.Value One-way Analysis of Variance (ANOVA)
Salivary pH	6.74 $\pm$ 0.28	6.78 $\pm$ 0.26	6.84 $\pm$ 0.30	0.635

Discussion

According to reports from the World Health Organization, the oral microbiota plays a vital role in maintaining health or contributing to disease (9). Although changes in the composition of oral microbiota in patients with celiac disease have previously been described, their pattern and clinical significance had not been clearly defined (22). The aim of the present study was to comparatively evaluate dental caries, the pattern of cariogenic microbial pathogens, and salivary pH in patients with celiac disease consuming gluten-containing or gluten-free diets in comparison with healthy individuals.

The findings of the present study demonstrated a statistically significant difference in the *Streptococcus mutans* load among the study groups. According to the results, the microbial load of *Streptococcus mutans* in patients with celiac disease consuming a gluten-containing diet was significantly higher than in patients adhering to a gluten-free diet, and in both celiac groups, the bacterial load was significantly higher compared with the control group. Consistent with the findings of the present study, Acar et al., who investigated the prevalence of enamel defects in patients with celiac disease and healthy individuals, reported a significantly higher *Streptococcus mutans* bacterial load in patients with celiac disease (5). In contrast to the present study, Francavilla et al. found no difference in salivary streptococcal load between children with celiac disease and the control group (6). Similarly, the study by Shteyer et al., which evaluated oral health status and salivary properties in relation to a gluten-free diet in children with celiac disease, reported no significant differences between groups in terms of salivary *Streptococcus mutans* counts (22). Moreover, Liu et al., in a study assessing salivary gland involvement and oral health in patients with celiac disease, found no statistically significant difference in salivary *Streptococcus mutans* counts between patients and healthy individuals (9). The discrepancies observed among studies may be attributed to differences in sampling methods, bacterial culture and enumeration techniques, age groups, oral hygiene habits, dietary patterns, environmental characteristics, salivary immune factors, and salivary composition.

In the present study, the mean *Lactobacillus acidophilus* load was evaluated among patients with celiac disease following gluten-free and gluten-containing diets, as well as healthy controls. The results indicated a statistically significant difference in *Lactobacillus acidophilus* bacterial load among the three groups. Although no significant difference was observed between the two celiac groups, both

demonstrated significantly higher bacterial loads compared with the control group. In agreement with these findings, Di Cagno et al., in a study investigating microbiota and metabolism in 19 children with celiac disease under a gluten-free diet, reported that the proportion of strains identified as *Lactobacillus acidophilus* differed significantly between patients with celiac disease and healthy individuals (23). Similarly, Nadal et al. demonstrated that the total proportion of *Lactobacillus* bacteria in patients with active celiac disease was significantly higher than in asymptomatic patients and healthy controls (24). Ercolini et al. also reported that changing dietary patterns from gluten-containing to gluten-free diets, or vice versa, led to alterations in *Lactobacillus acidophilus* bacterial profiles in patients (25). In a study conducted by Tian et al. evaluating salivary gluten degradation and oral microbial load in healthy individuals and patients with celiac disease, higher levels of *Lactobacillus* species were observed in the saliva of patients with celiac disease (26). Conversely, Francavilla et al. reported no difference in *Lactobacillus* load in the saliva of children with celiac disease compared with controls (6). This inconsistency may be attributed to differences in age groups, as the present study evaluated adults aged 20–40 years, whereas Francavilla et al. focused on pediatric populations. Similarly, Liu et al. found no statistically significant difference in salivary *Lactobacillus* counts between patients with celiac disease and healthy individuals (9). Findings regarding the role and behavior of this bacterium remain inconsistent across studies. Kreutz et al. reported lower levels of *Lactobacillus acidophilus* among patients (27), while Shteyer et al. found no difference in salivary *Lactobacillus* load between groups (22). In contrast, Acar et al. reported significantly lower *Lactobacillus* bacterial load in children with celiac disease compared with healthy children (5). *Lactobacillus acidophilus* is recognized as one of the most important microorganisms involved in gluten degradation in saliva (23). Species of *Lactobacillus* are also considered part of the main oral microflora (28). Factors such as probiotic consumption, geographical variations, population characteristics, and the volume of collected saliva samples may influence the level and mean load of *Lactobacillus acidophilus* (29).

The present study demonstrated that the mean DMFT index in patients with celiac disease consuming a gluten-containing diet was significantly higher than in patients adhering to a gluten-free diet, and both groups exhibited significantly higher DMFT values compared with healthy controls. In agreement with these findings, Privolou et al., in a comparative study evaluating enamel defects and dental caries in children and adolescents with and without celiac disease, reported higher DMFT values among patients consuming gluten-containing diets (30). Bulut et al. also reported a significantly higher prevalence of dental caries in children with long-standing celiac disease compared with those newly diagnosed (31). A systematic review further indicated that DMFT values in patients with celiac disease were nearly twice as high as those observed in healthy individuals (32). Several previous studies have similarly reported higher DMFT indices among patients with celiac disease (33, 34). Consistent with the present findings, Damla et al. reported a statistically significant difference in DMFT values between children with celiac disease and healthy controls (20). Likewise, Cantekin et al. demonstrated significantly higher DMFT indices in patients with celiac disease compared with healthy individuals (35). In contrast, some studies have reported no difference in DMFT values between patients with celiac disease following gluten-containing or gluten-free diets. Albuquerque et al., in a systematic review, found no significant difference

in DMFT between patients with celiac disease and healthy individuals (36). Amirabadi et al. also reported no difference in DMFT indices between children with celiac disease and healthy controls (21). Acar et al. similarly observed comparable DMFT values in both groups (5). In certain studies, a lower prevalence of dental caries has even been reported among patients with celiac disease. It has been suggested that this lower prevalence may be due to strict dietary control and improved dental care in these patients, resulting in reduced cariogenic exposure (37). Some evidence indicates that celiac disease may also influence the eruption timing of permanent teeth, which may affect the duration of exposure to cariogenic conditions and consequently alter caries patterns (38). Nutritional deficiencies such as vitamin D, calcium, and zinc deficiency have also been proposed as potential contributing factors to oral and dental manifestations in patients with celiac disease (39). Differences in lifestyle, dietary composition, oral health literacy, effectiveness of oral hygiene education programs, and oral hygiene behaviors are among variables that may influence DMFT indices. Since evaluation of these factors was beyond the scope of the present study, failure to assess and control them may partly explain differences between the findings of this study and previous reports (40).

In the present study, no statistically significant difference was observed in salivary pH between patients with celiac disease consuming gluten-containing diets, those following gluten-free diets, and healthy controls. These findings are consistent with those reported by Shteyer et al., who observed no difference in salivary pH among children with celiac disease (22). Similarly, Acar et al. found no significant difference in salivary pH between children with celiac disease and healthy controls (5). Saliva may serve as a valuable indicator for predicting dental manifestations in patients with celiac disease. In contrast to the present findings, Damla et al. reported a significant difference in salivary pH between children with celiac disease and healthy individuals (22). Additionally, Baliga et al. demonstrated that salivary pH was more alkaline in patients with generalized chronic gingivitis, whereas it was more acidic in patients with generalized chronic periodontitis compared with controls (17). It appears that salivary pH in patients with long-standing celiac disease may remain affected despite adherence to a gluten-free diet. Variations among study findings may be attributed to differences in clinical characteristics, salivary flow rate, salivary sugar content, buffering capacity, timing and method of saliva collection, and pH measurement techniques (9). Overall, the present study indicates that celiac disease can influence the oral environment and salivary microbial composition, thereby increasing the risk of dental caries. Higher loads of *Streptococcus mutans* and *Lactobacillus acidophilus* in patients with celiac disease—particularly those consuming gluten-containing diets—represent important changes that should be considered by dentists and gastroenterologists. Furthermore, the elevated DMFT index in these patients highlights the need for early preventive and therapeutic interventions.

Conclusion

The results of the present study indicate that dietary gluten plays a significant role in altering the oral microbial environment in patients with celiac disease. Increased salivary loads of *Streptococcus mutans* and *Lactobacillus acidophilus*—particularly among patients consuming gluten-containing diets—were associated with a higher risk of dental caries. Adherence to a gluten-free diet was found to be effective

not only in controlling gastrointestinal symptoms but also in reducing cariogenic bacterial load and improving oral health outcomes. The significantly higher DMFT index observed in patients with celiac disease highlights the necessity of early preventive dental care and continuous oral health monitoring in this population. These findings emphasize the importance of interdisciplinary collaboration between gastroenterologists and dental professionals in the comprehensive management of patients with celiac disease. Implementation of preventive oral health programs, alongside strict dietary control, may contribute to improved quality of life and reduced oral complications in affected individuals.

Declarations

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Authors' contributions

YB and DN initiated and designed the study. MA and EVM performed data processing and analysis. DN, MA, and MR-GH performed validation of results and YB and DN were responsible for creating and revising all figures. The initial draft of the article was written by YB, MA, and RF. Final revisions and editing were performed by YB, EV-M, MA, RF, MR-GH and DN

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Ethics approval and consent to participate

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the Institutional Review Board and the Declaration of Helsinki of 1964. All authors declare that written informed consent was obtained from all study participants. Also, the study received ethical approval (ethics code: IR.MUQ.REC.1404.088) from the Ethics Committee of the Vice-Chancellor for Research and Technology, Qom University of Medical Sciences.

Consent for publication

The complete investigative group in this considers donating their personal assent for publication.

Data Availability

The authors declare that the data of this study are available and can be provided upon reasonable request from the corresponding author of the article.

Competing interests

No competing interests include financial and non-financial, business or family interests

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Figures



Figure 1

Selective Blood Agar culture medium for *Streptococcus mutans*



Figure 2

BHI Broth liquid culture medium for primary growth enhancement of *Streptococcus mutans*



Figure 3

Anaerobic jar with candle for incubation of *Lactobacillus* plates



Figure 4

MRS Agar selective culture medium for *Lactobacillus*

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