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Article

Efficacy of Probiotic Mouthwash Comparing to Conventional Types on Salivary PH and Cariogenic Microbiota Among Patients with Fixed Orthodontic Appliance

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Abstract

Background/Objectives: Recent research indicate that probiotics positively influence oral health and impact oral micro-ecology. This study evaluates the effectiveness of probiotic mouthwash toward to *Streptococcus mutans* and *Lactobacillus* spp in oral cavity of patients with fixed orthodontic appliances comparing to conventional mouthwashes through count bacteria colonies and measurement of salivary pH. **Methods:** Collection of salivary and plaque samples from total 30 patients (15 males and 15 females), aged 14-21 years were done for colony counting of the caries related microorganisms and measurement of salivary ph. The study was included three groups with three treatment times as following: baseline, after two weeks, after one month, Group1: used Orthin Kin mouthwash, Group 2: Corsodyl mouthwash, Group 3: Probiotic mouthwash. **Results:** (33.3%, 96.6%) of samples were positive for *S. mutans* in male and female respectively while lactobacillus were (100%) in both. On the other hand, (93.33% ,3.33%) of plaque and saliva samples were positive for *S. mutans* respectively while, lactobacillus spp (100.0 %) of plaque and saliva samples. The treatment with probiotic, Orth Kin and Corsodyl mouthwashes were reduced *S. mutans* in plaque compared to baseline with significant difference at $p < 0.05$ between probiotic and both orthoKin and Corsodyl. While in saliva, treatment with probiotic showed increased *lactobacillus* spp level after month compared with baseline. However, the salivary PH showed there was no significant difference between studied groups and baseline. **Conclusion:** Using the Probiotic mouthwash for two weeks and a month gave effective results than others. However, the salivary pH did not change during the treatment in studied groups.

Keywords: probiotic mouthwash; orthodontic appliance; chlorhexidine; Orthkin; Corsodyl; *S. mutans* and *Lactobacillus* spp; salivary PH

1. Introduction

In modern orthodontics, fixed appliance therapy is a crucial component. Even though orthodontic treatment has many advantages for the patient, fixed appliances can harm the dentition and supporting tissues if proper and consistent oral hygiene is not maintained [1].

The primary cause of tooth demineralization and the development of cavities is plaque buildup and retention in the vicinity of fixed orthodontic appliance [2]. Plaque retention can be managed in a variety of ways, including brushing teeth and using mouthwashes containing fluoride and

chlorhexidine. However, because it is a chemical substance, it typically has negative consequences like dry mouth and tooth discoloration [3,4]. Antibiotics, antimicrobial medication with chlorhexidine, fluoride, and other biological techniques are typically employed to manage plaque. At the moment, these techniques can reduce the caries infection but never completely eradicate it [5].

Probiotics are good microorganisms that can help the host's micro-ecological balance [6]. The Food Agricultural Organization/World Health Organization described it as live microorganisms that, when given in sufficient quantities (in food or as a dietary supplement), promote the host's health by enhancing the digestive tract's microbiological balance [7]. Although probiotics have long been employed in fermented foods, its promise as a nutritional therapy has just lately been officially recognized [8].

The majority of probiotic bacteria are found in yoghurt products and probiotic drinks, and they are classified under the genera *Lactobacillus* or *Bifidobacterium*. According to recent research, probiotics improve oral health and have the ability to modify the protein makeup of dental plaque, which in turn can impact the oral micro-ecology [9]. Research on the anti-plaque and anti-caries effects of probiotics has increased as a result of this [10].

Lactobacilli probiotics produce antifungal compounds such as fatty acids and bacteriocins, which exhibit bactericidal and bacteriostatic effects, along with antimicrobial agents including organic acids and hydrogen peroxide [11,12] assert that *S. mutans* is the most recognized pathogenic bacterium responsible for dental caries due to its acidogenicity, aciduric characteristics, and ability to generate an extracellular matrix that is the fundamental component of dental plaques and biofilms [13]. While numerous research on the use of probiotics have shown that *Lactobacillus* inhibits *S. mutans* in dental plaque, there aren't many that compare the use of probiotics with chemical mouthwashes like Ortho Kin and Corsodyl over the same time period. We believe that prolonged usage of probiotics produces superior benefits than the two chemical mouthwashes (Corsodyl and Orthokin). This study aims to compare the efficacy of Orthokin and Corsodyl mouthwashes with probiotic mouthwashes on *S. mutans* and *Lactobacillus* spp in the oral cavity by counting the number of bacterial colonies and measuring the pH of saliva.

2. Materials and Methods

2.1. Sample Collection

At the private orthodontic clinic in Baghdad, Iraq, 30 patients (15 males and 15 female) between the ages of 14 and 21 who were receiving treatment with fixed orthodontic appliances, the goal of the study was explained to the participants, and informed consent for participation was obtained from all subjects involved in the study. Ethical approval for this study (Ethical Committee No. 5) was provided by the Ethical Committee in collage of dentistry, Dijlah university, Baghdad, Iraq, on 17 October 2024.

The following criteria were used to select patients: the patient had to have a complete permanent dentition, be free of caries, systemic diseases, or allergic conditions, brush their teeth twice a day, and not have taken any medication, including corticosteroids or systemic antibiotics, for at least four weeks prior to the study. The materials utilized were Corsodyl mouthwash (containing chlorhexidine, Germany), Orthokin mouthwash (containing fluoride, Spain), and Biorepair probiotic mouthwash (Italy) Figure 1.



Figure 1. The mouthwashes that used in study (Biorepair Probiotic, Corsodyl and OrthoKin).

Following the manufacturer's recommendations, the patients were told to use the mouthwash after cleaning their teeth. The samples were split up into three groups, and each group received three different treatment times: baseline, two weeks later, and one month later (each group had 10 patients) as in Figure 2. Ortho Kin mouthwash was used by Group 1, Corsodyl mouthwash by Group 2, and Biorepair probiotic mouthwash by Group 3 (10 patients). At least an hour after the meal, between 4-5 pm, salivary and plaque samples were collected. Using a sterile instrument, a swab was taken between the orthodontic brackets on the buccal and labial surfaces of the upper teeth close to the bracket base in order to capture the plaque, while for saliva collection, 2 ml was collected then both samples added into a separated sterile, plastic capped tube with a normal saline to have tenfold dilutions and transferred to the microbiology laboratory.

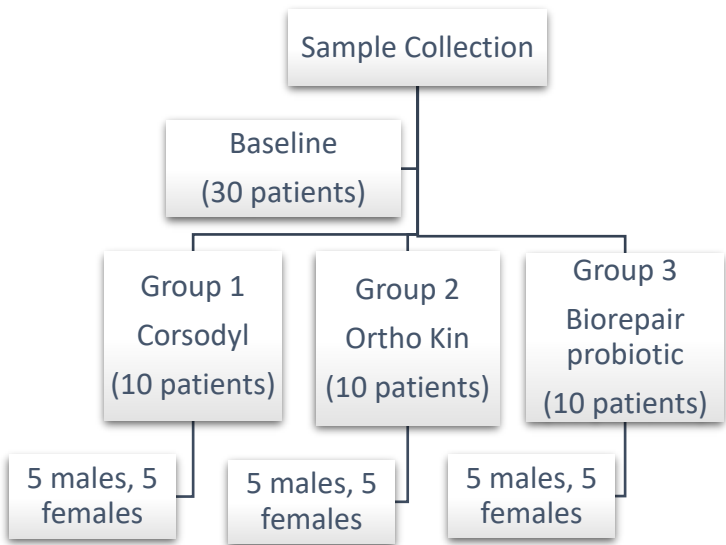


Figure 2. Flowchart of studies groups (Same in saliva and plaque samples).

2.2. Assessment of *S. mutans* and *Lactobacillus* spp Colony Counts

For the purpose of full colony counting of related microorganisms (*S. mutans* and *Lactobacillus* spp), the special selective media were used (Mitis-Salivarius-Bacitracin (MSB) agar (HiMedia, Indian origin Bioscience company) for *S. mutans* and De Man–Rogosa–Sharpe (MRS) agar (HiMedia, Indian origin Bioscience company) for *Lactobacillus* spp. *Lactobacillus* spp colonies were counted by pouring 1×10^1 on plates contain MRS agar and incubated anaerobically at 37°C for 3 days, the isolates were on the basis of colony morphology, gram staining (rod-shaped gram positive bacteria, non-spore forming,) and basic biochemical tests (catalase-positive and oxidase-negative).

Same procedure was followed for *S. mutans*, one ml of 1×10^1 dilute was poured on plates contain MSB agar at 37 C° in a microaerophilic atmosphere (5% O2, 10% CO2) for 24 hr.

2.3. Measurement of Salivary pH

A pH meter was used right away to assess the salivary pH (Radiometer, Crawley, UK). Freshly made pH 7 buffers were used to calibrate the pH meter, and while not in use, the electrode was kept immersed in double-distilled water. Following pH analysis, the electrode tip was once more cleaned with a mild stream of distilled water before being dipped in double-distilled water.

2.4. Statistical Analysis

All of the study's parameters were examined in order to find the effects of different factors using the Statistical Analysis System-SAS-18 application. Significant mean comparisons were also made using the LSD test (Analysis of Variation-ANOVA), which measures the least significant difference at $p \leq 0.05$.

3. Results

3.1. The Distribution of Bacteria

The distribution of *S.mutans* and *Lactobacillus* in plaque and saliva represented as: distribution of bacteria according the gender , distribution of bacteria according to the plaque and saliva samples, and distribution according the age as in Figure 3.

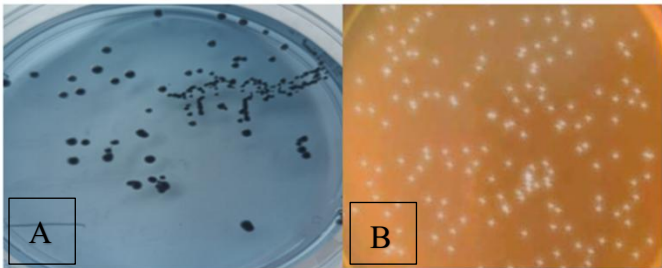


Figure 3. A: *S. mutans* on MSB agar. B: *Lactobacillus* spp on MRS agar in plaque and saliva sample.

3.2. Distribution of Bacteria According to Gender

The results of this study revealed that (33.3%) of samples was positive for *S. mutans* in male and (96.6%) in female, while *lactobacillus* spp showed (100%) in male and female as in Figure 4.

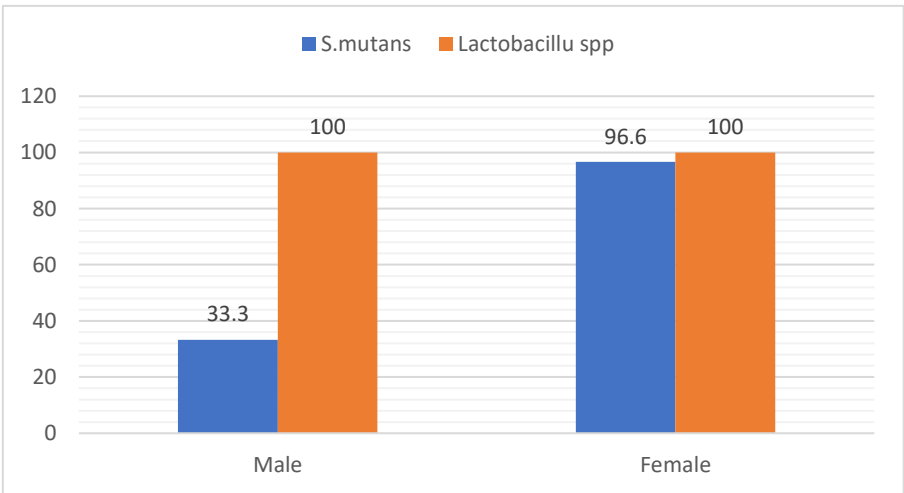


Figure 4. Distribution of bacteria according to gender.

3.3. Distribution of Bacteria in Samples

The results of this study revealed that (93.33%) of plaque samples was positive for *S. mutans* , while (3.33 %) of saliva sample was positive *S.mutans* , However the results reported that (100.0 %) of plaque and saliva samples was positive for *lactobacillus* spp as in Figure 5.

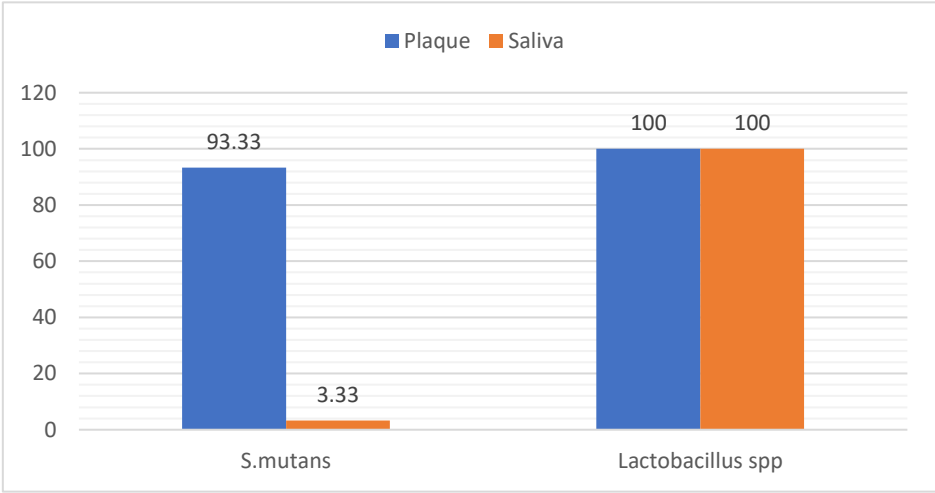


Figure 5. Distribution of bacteria in saliva and plaque.

3.4. Distribution of Bacteria According the Age

The findings revealed all subjects showed (100%) positive for *lactobacillus* spp of plaque and saliva. Moreover, the saliva sample in subjects age < 15 year showed no *S. mutans*, while 6.6% of saliva sample in age > 15 year showed positive for *S. mutans*. On the other hands, 93.3 % of plaque samples in subjects with age > 15 year were positive of *S. mutans*. Finally, *S. mutans* was recorded 100% in plaque samples in subjects with age <15 year as in Figure 6.

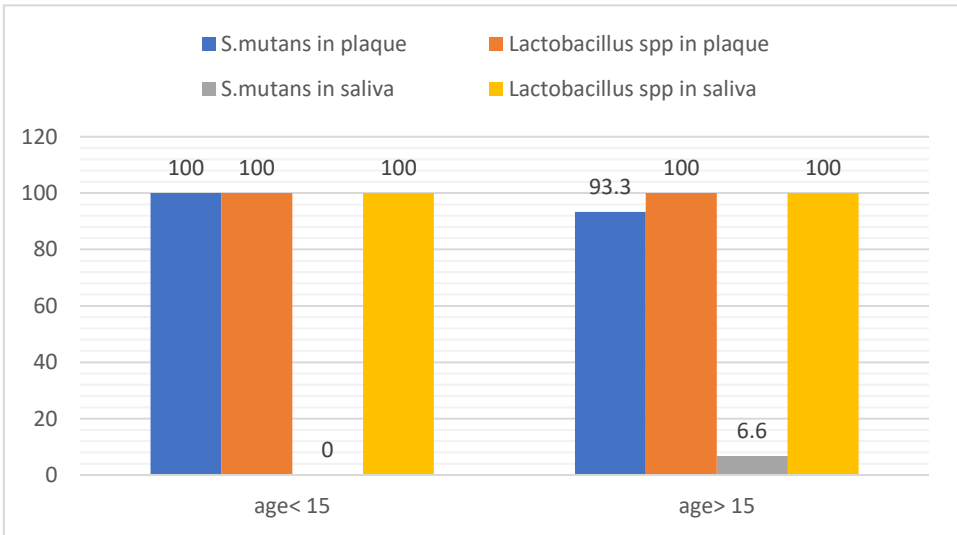


Figure 6. Distribution according the age.

3.5. Distribution of Bacteria in Studied Groups

The finding showed that the treatment with probiotic, Orth Kin and Corsodyl for a month showed a good effect against *S. mutans* in plaque (37.00 ± 1.41 , 79.00 ± 0.00 , 69.00 ± 0.00) respectively compared to baseline (95.75 ± 24.94) with significant difference at $p < 0.05$, with no significant difference recorded between Orth Kin and Corsodyl, but there was a significant difference between

probiotic and both Kin and Corsodyl. The findings after two week of the treatment with probiotic, Orth Kin and Corsodyl were (61.50 ±38.89, 88.50 ±0.70, 70.50 ±0.70) respectively reported a significant difference with baseline (95.75±24.94) at p< 0.05, but no significant difference between probiotic and Corsodyl, moreover there was a significant difference between orthokin and both probiotic and Corsodyl at p< 0.05 as in Table 1. Moreover, only one saliva sample was positive for *S. mutans* recorded in male patient age > 15, this was statistically ignored and not taken into consideration in current study.

Table 1. *S. mutans* in plaque (CFU X10⁻¹).

Treatment	Mean± SD
	<i>S.mutans</i> in Plaque
Baseline	95.75±24.94 a
Probiotic after two week	61.50 ±38.89 b
OrthoKin after two week	88.50 ±0.70a
Corsodyl after two week	70.50 ±0.70 b
Probiotic after a month	37.00 ±1.41 c
OrthoKin after a month	79.00 ±0.00 b
Corsodyl after a month	69.00±0.00 b

*The similar letters refer to no significant difference at p<0.05 *The different letters refer to significant difference at p<0.05.

For *Lactobacillus* spp in plaque; the findings were recorded that after a month of the treatment with probiotic there was no significant difference with baseline; while both Orth Kin and Corsodyl (52.50±74.24; 79.50±37.47) respectively showed a significant difference at p<0.05 with baseline (124.50 ±4.94); from the other side the probiotic treatment reported the highest value 127±20.01. In addition; for the treatment after two weeks there was significant difference between all treatment with no treatment, but no significant difference between probiotic and Corsodyl treatment at p<0.05 also the probiotic treatment reported the highest value 111.00 ±28.70.

While in saliva sample the treatment after a month with probiotic, Orth kin and Corsodyl (130.50±0.05, 76.00±0.00, 46.00±65.05) respectively showed significant difference with baseline (128 ±29.69), the probiotic treatment recorded the highest value 130.50±0.05 while after two week no significant differences between all treatment with baseline except treatment with OrthoKin 83 ±0.00 showed significant difference with no treatment 128 ±29.69 at p<0.05 as well as the probiotic treatment reported the highest value 117.50 ±0.05 compared with other treatments as in Table 2.

Table 2. *Lactobacillus* spp in plaque and saliva (CFU X10⁻¹).

Treatment	Mean± SD	Mean± SD
	<i>Lactobacillus</i> spp in Plaque	<i>Lactobacillus</i> spp in Saliva
Baseline	124.50±4.94 a	128 ±29.69 b
Probiotic after two week	111.00 ±28.70 b	117.50 ±0.05 b
OrthoKin after two week	90.00±0.00 c	83 ±0.00 c
Corsodyl after two week	88.00±39.59b	100.00 ±1.41 b
Probiotic after a month	127±20.01 a	130.50±0.05 a
OrthoKin after a month	52.50±74.24 d	76.00±0.00 c
Corsodyl after a month	79.50±37.47 c	46.00±65.05 d

*The similar letters refer to no significant difference at $p<0.05$. *The different letters refer to significant difference at $p<0.05$.

3.6. Salivary pH

The results showed there was no significant difference in saliva PH in treatment and no treatment period as showed in Table 3.

Table 3. Salivary pH in studied group.

Treatment	Mean± SD
Without Treatment	7.1±0.20 a
Probiotic after two week	7.15 ±0.03 a
OrthoKin after two week	7.3±0.00 a
Corsodyl after two week	6.95±0.05 a
Probiotic after a month	7.1 ±0.05 a
OrthoKin after a month	7.1±0.00 a
Corsodyl after a month	7.1±0.1 a

* The similar letters refer to no significant difference at $p<0.05$.

4. Discussion

The results were in agreement with those of Toi et al [14] who examined 83 subjects ranging in age from 3 to 17 years, 45 of whom were female (54.2%) and 38 of whom were male (45.8%). The samples showed positive levels of *S. mutans* in both saliva and plaque. Additionally, the findings were in agreement with those of Khalaf et al. [15] who examined 135 children aged 3 to 10 years and discovered that *S. mutans* was more prevalent in dental plaque. According to Mummolo et al. [16], 40 people (aged 20.4 ± 1.7 years) had hazardous salivary levels ($CFU/ml>10^5$) of both *Lactobacilli* and *S. mutans* out of the 80 participants (46 men and 34 females) in the study.

Conversely, using a molecular technique like as PCR analysis, Lee et al. [17] found *S. mutans* in 56.8% of plaque samples and 79.7% of saliva samples. Interestingly, children's saliva samples had a much lower percentage of *S. mutans* (59.1%) than did those from teenagers (88.0%) and adults (88.9%). On the other hand, *S. mutans* was found far more frequently in the plaque samples taken from children (86.4%) compared to those taken from adolescents (56.0%) and adults (33.3%), suggesting that there is a trend for dental caries to become more common as people age. Interestingly, for each of the three age groups, the value was marginally greater in the female population than in the male population.

Furthermore, our study found that the distribution of bacteria in saliva and plaque varied amongst studies, but this variation was consistent with [18] who collected different samples from dental plaque (DP), saliva swab (SS), and whole saliva (WS). The SS technique noticed a significantly lower value of cfu/mL in comparison to the DP and WS methods ($P < 0.05$). Nonetheless, the outcomes of the WS and DP approaches were comparable ($P > 0.05$). Using the WS (100%) and SS (14.3%) procedures, only seven of the 15 participants in the first sequence of methods had a positive count for *Lactobacillus* Spp. Based on the study by Seki et al. [19] which showed that a greater number of locations for dental plaque collection greatly rises, samples from all tooth surfaces were employed for the dental plaque collection method. Nevertheless, occlusal plaque had greater *S. mutans* counts than saliva samples, indicating that plaque might be a more accurate way to identify high *S. mutans* levels than saliva. This disparity might be explained by a greater supply of *S. mutans* in the oral plaque, which is the bacterium's principal home. Saliva may also be a similar proxy because *S. mutans* isolates from plaque into it during normal salivary clearance. According to Nguyen et al [20] saliva and occlusal plaque can be regarded as trustworthy sources for *S. mutans* identification and quantification, irrespective of the state of the teeth.

Furthermore, the results of Treatment were agreed with several studies as the results of saliva samples were obtained from all 58 pediatric patients without any systemic medical conditions on the day (T0), one month (T1), three months (T2), and six months (T3) following general anaesthesia. by Sakaryalı et al. [21] were agreed with our findings, even after the probiotic was stopped, the levels of *S. mutans* decreased in both groups over the course of the follow-up period. This decrease was not statistically significant, though. However, a statistically significant difference was observed as T3 levels of *Lactobacillus* spp. increased during the follow-up period in both groups, although the probiotics group experienced a statistically significant decrease during the first month of follow-up. Similar findings were reported by Badri and his colleague in 2021 [22], who discovered that 54 participants, ages 8 to 12, were divided into three groups at random, with 18 youngsters in each group. Children in the CHX group were instructed to utilize CHX mouthwash bi-daily, while those in the probiotic group had one probiotic lozenge (*Biogaia prodentis*) daily. The results showed that both the CHX mouthwash and the probiotic lozenges dramatically decreased the number of *S. mutans*. Keller et al. [23] found that the regrowth of salivary *S. mutans* levels was unaffected by the daily oral dose of *Lactobacillus reuteri*. According to a recent study, probiotic toothpaste and curd effectively decreased the quantity of streptococcus mutans in orthodontic patients [24].

Finally, our findings indicated no significant differences between groups in salivary PH, which contradicts other research, including Badri et al [22], who demonstrated that probiotic lozenges and CHX mouthwash dramatically decreased plaque index and *S. mutans* count while increasing salivary pH values. However, no statistical differences between the effect of and CHX mouthwash and probiotic lozenges in PH value. Different studies found there was increasing in salivary PH as According to Srivastava et al. [25], when compared to regular curd, probiotic curd significantly increased salivary pH and decreased salivary *S. mutans* count from baseline to seven days. Probiotic curd is preferable to normal curd because it raised salivary pH after consumption. In a different study, Hoorizad et al. [26] examined the effects of probiotic and non-probiotic yoghurt on orthodontic patients' salivary pH and found that probiotic yoghurt can raise salivary pH, creating an environment that is conducive to the remineralization of tooth substance structures. This suggests that curd does not present a caries risk despite its acidic nature [27]. The limitations of the current study represented by the limited number of samples during the study period due to lack of commitment, the totals received the prescribed treatments, in addition to the fact that most of them did not attend the clinic at the specified time to take the samples.

5. Conclusions

Using the Probiotic mouthwash for two weeks and a month gave effective results than Orthkin and Corsodyl mouthwashes in which *S. mutans* level was reduced and at the same time *Lactobacillus* spp level was increased in group that treated with probiotic mouthwash compared with other mouthwashes. However, the salivary pH of the did not change during the treatment in studied groups in during study period.

Author Contributions: Conceptualization, W.K.A.-L. and N.N.A.; methodology W.K.A.-L.; formal analysis, W.K.A.-L.; validation, W.K.A.-L. and N.N.A., investigation, N.N.A.; resources, W.K.A.-L.; data curation, L.A.A.; writing—original draft preparation, W.K.A.-L.; writing—review and editing, W.K.A.-L. and N.N.A.; visualization, L.A.A.; supervision, L.A.A.; funding acquisition, W.K.A.-L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of College of Dentistry, Dijlah University (ref. [number 5] on 17 October 2024).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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