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Comparative Antimicrobial Efficacy of Amine Fluoride and Sodium Fluoride Mouthwashes on Cariogenic Bacterial Colonization Around Orthodontic Brackets: A Prospective Randomized Clinical Study

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Abstract: This randomized controlled study compared the antimicrobial effects of amine fluoride and sodium fluoride mouthwashes in 30 patients undergoing fixed orthodontic treatment. Participants were assigned to amine fluoride, sodium fluoride, or routine oral hygiene alone (n = 10 per group). Plaque samples from the maxillary first premolar region were collected at baseline and after one month to quantify *Streptococcus mutans* and *Lactobacillus acidophilus*. Both mouthwashes significantly reduced counts of both species from baseline ($P < 0.001$) and compared with routine oral hygiene alone. The difference between fluoride formulations was statistically nonsignificant ($P > 0.05$). Both mouthwashes may serve as adjuncts to routine oral hygiene during fixed orthodontic treatment.

Keywords: *Fixed Orthodontic Appliances, Amine Fluoride, Sodium Fluoride, Streptococcus mutans, Lactobacillus acidophilus.*

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I. INTRODUCTION

Fixed orthodontic appliances create additional plaque-retentive surfaces that alter the oral microbial environment and promote biofilm accumulation. The presence of brackets, archwires, and ligatures hinders effective plaque removal, leading to increased colonization by cariogenic microorganisms, particularly Streptococcus mutans and Lactobacillus acidophilus. These microorganisms play a central role in the initiation and progression of enamel demineralization, white spot lesions (WSLs), and dental caries, which remain among the most common iatrogenic complications of fixed orthodontic treatment. WSLs may develop within the first few weeks after appliance placement and can adversely affect both esthetic and functional treatment outcomes. Consequently, maintaining optimal oral hygiene throughout orthodontic therapy is essential to preserve enamel integrity and ensure long-term treatment success. [1-4]

Mechanical plaque control through regular tooth brushing and interdental cleaning remains the cornerstone of oral hygiene maintenance during fixed orthodontic treatment. However, the complexity of fixed appliances frequently limits the effectiveness of mechanical cleaning, particularly in patients with suboptimal oral hygiene practices or poor compliance. Therefore, adjunctive chemical plaque control measures are routinely recommended as part of preventive orthodontic care. Among these, fluoridated mouthwashes have gained widespread acceptance because they combine antimicrobial activity with the ability to enhance enamel remineralization. Fluoride inhibits bacterial metabolism and acid production while promoting the formation of fluorapatite, thereby increasing enamel resistance to acid-induced demineralization. Daily use of fluoride-containing adjuncts has been shown to reduce the incidence of WSLs during orthodontic treatment. [5-8]

Several fluoride formulations are commercially available, with sodium fluoride and amine fluoride being among the most frequently prescribed for orthodontic

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patients. Although both formulations exhibit anticariogenic properties, their mechanisms of action differ. Sodium fluoride primarily acts by increasing the availability of fluoride ions to enhance enamel remineralization and inhibit demineralization. In contrast, amine fluoride possesses surface-active properties that improve fluoride retention on tooth surfaces and facilitate prolonged interaction with the dental biofilm, potentially enhancing its antimicrobial effect. These biological differences suggest that the two formulations may differ in their ability to suppress cariogenic bacterial colonization during fixed orthodontic treatment. Recent clinical evidence has demonstrated that amine fluoride mouthwash is effective in reducing white spot lesions during orthodontic therapy, further supporting its role as an adjunctive preventive agent. [9-11]

Previous studies have demonstrated that fluoride mouthwashes reduce plaque accumulation, decrease cariogenic bacterial counts, and minimize the risk of enamel demineralization in orthodontic patients. However, most published investigations have evaluated individual fluoride formulations or compared fluoride mouthwashes with chlorhexidine rather than directly comparing different fluoride formulations. Consequently, evidence regarding the relative antimicrobial efficacy of amine fluoride and sodium fluoride mouthwashes against Streptococcus mutans and Lactobacillus acidophilus during fixed orthodontic treatment remains limited. Furthermore, recent consensus statements and systematic reviews continue to emphasize the need for high-quality randomized clinical trials evaluating preventive strategies for WSLs during orthodontic treatment. [4,7,12-14]

Therefore, the present prospective randomized controlled clinical study was undertaken to compare the antimicrobial efficacy of amine fluoride and sodium fluoride mouthwashes in reducing Streptococcus mutans and Lactobacillus acidophilus colonization around orthodontic brackets after one month of use in patients undergoing fixed orthodontic treatment. It was hypothesized that both fluoride formulations would significantly reduce cariogenic bacterial colonization compared with routine oral hygiene alone, while differences in their antimicrobial efficacy, if present, could provide clinically relevant evidence for selecting an appropriate fluoride mouthwash during fixed orthodontic treatment

II. METHODS

➢ *Study Design*

This prospective, randomized, controlled in vivo clinical study was designed to compare the antimicrobial efficacy of amine fluoride and sodium fluoride mouthwashes in reducing cariogenic bacterial colonization around orthodontic brackets during fixed orthodontic treatment.

Written informed consent was obtained from all participants before enrollment, and the study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

➢ *Sample Size Calculation*

The sample size was calculated using G*Power software (Version 3.1.9.7) based on data from previous studies evaluating the antimicrobial effect of fluoride mouthwashes in orthodontic patients. Assuming a significance level (α) of 0.05, a statistical power of 80%, and an anticipated large effect size, the minimum required sample size was six participants per group. To improve statistical reliability and compensate for possible dropouts, ten participants were recruited into each group, resulting in a total sample size of 30 participants.

➢ *Participant Selection*

Thirty patients undergoing fixed orthodontic treatment were recruited consecutively from the outpatient clinic of the Department of Orthodontics.

➢ *Inclusion Criteria*

Patients aged 15 to 30 years undergoing fixed orthodontic treatment.

- Bonded with Damon Q metal brackets.
- Fully erupted maxillary first premolars.
- Permanent dentition from the maxillary right first molar to the left first molar.
- Good general health.
- Willingness to participate and provide written informed consent.

➢ *Exclusion Criteria*

Presence of enamel hypoplasia, developmental enamel defects, or existing white spot lesions.

- Restorations involving the maxillary first premolars.
- Congenitally missing, partially erupted, or impacted maxillary first premolars.
- Patients receiving systemic antibiotics within one month before enrollment.
- Presence of active periodontal disease.
- History of allergy to fluoride mouthwashes.
- Debonding of brackets during the study period.
- Failure to comply with the prescribed oral hygiene protocol.

➢ *Randomization*

Eligible participants were randomly allocated into three equal groups (n = 10 per group) using a web-based online randomization table. The random allocation sequence was generated before participant enrollment, and eligible participants were assigned directly according to the generated allocation sequence.

- *The Participants were Allocated as Follows:*

✓ Group I (Control): Routine oral hygiene instructions without adjunctive mouthwash.
✓ Group II (Amine fluoride): Routine oral hygiene instructions with amine fluoride mouthwash (Amflor®).

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- ✓ Group III (Sodium fluoride): Routine oral hygiene instructions with sodium fluoride mouthwash (Phosflur®).

➢ *Clinical Protocol*

All participants received standardized oral hygiene instructions before appliance placement. They were instructed to brush twice daily using a fluoridated toothpaste and an orthodontic toothbrush and to rinse thoroughly with water after meals.

Participants in Groups II and III were instructed to rinse with 10 mL of the allocated mouthwash twice daily for 1 minute, once after morning tooth brushing and once before bedtime. Participants were instructed not to swallow the mouthwash and to avoid eating or drinking for at least 30 minutes after rinsing.

Compliance with the prescribed mouthwash regimen was assessed at the one-month follow-up through verbal confirmation and inspection of the returned mouthwash bottles.

➢ *Plaque Sample Collection*

Supragingival plaque samples were collected from the maxillary right first premolar immediately before initiation of the intervention (baseline) and after one month.

The tooth was isolated with cotton rolls and gently air-dried. A sterile size 40 absorbent paper point was inserted into the gingival sulcus adjacent to the orthodontic bracket for approximately 10 seconds. The paper point was then transferred immediately into a sterile Eppendorf tube containing 1 mL of 10% thioglycollate broth supplemented with Tween 80 and transported to the microbiology laboratory for analysis.

➢ *Microbiological Analysis*

The plaque samples were vortexed for one minute to obtain a homogeneous bacterial suspension. Aliquots of the suspension were inoculated onto Mitis Salivarius Bacitracin (MSB) agar for selective isolation of Streptococcus mutans and de Man, Rogosa and Sharpe (MRS) agar for isolation of Lactobacillus acidophilus.

MSB agar plates were incubated aerobically at 37°C, whereas MRS agar plates were incubated anaerobically at 37°C for 48 hours. Following incubation, colony-forming units (CFU) were quantified using a digital colony counter. The microbiological assessment was performed using standardized laboratory procedures. The microbiologist performing colony counts was blinded to group allocation.

➢ *Outcome Measures*

The primary outcome measure was the change in $\log_{10}$-transformed colony-forming unit (CFU) counts of Streptococcus mutans and Lactobacillus acidophilus after one month of intervention.

The secondary outcome measure was the comparison of antimicrobial efficacy among the three study groups.

➢ *Statistical Analysis*

Statistical analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Bacterial colony counts were logarithmically transformed ($\log_{10}$ CFU) before statistical analysis to achieve approximate normality. Data distribution was assessed using the Shapiro–Wilk test.

Within-group comparisons between baseline and one-month bacterial counts were performed using the paired Student's t-test. Intergroup comparisons were carried out using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test for pairwise comparisons. Effect sizes were calculated using Cohen's d. A P value of <0.05 was considered statistically significant.

III. RESULTS

Thirty participants completed the study, with 10 participants in each group (control, amine fluoride, and sodium fluoride). No participant was lost to follow-up, and all samples were included in the final statistical analysis.

The Shapiro–Wilk test confirmed that $\log_{10}$-transformed Streptococcus mutans and Lactobacillus acidophilus colony counts followed a normal distribution in all study groups ($P > 0.05$). Therefore, parametric statistical tests were used for subsequent analyses (Table 1).

Within-group comparisons demonstrated a statistically significant reduction in both Streptococcus mutans and Lactobacillus acidophilus counts after one month of mouthwash use in the Amflor and Phosflur groups ($P < 0.001$). In contrast, the control group showed no significant change in bacterial counts during the study period (Table 2).

Baseline comparisons revealed no statistically significant differences among the three study groups for either microorganism, confirming adequate comparability before intervention ($P > 0.05$). However, significant intergroup differences were observed after one month for both Streptococcus mutans ($F = 37.09$, $P < 0.001$) and Lactobacillus acidophilus ($F = 42.64$, $P < 0.001$), indicating that adjunctive fluoride mouthwash therapy significantly reduced bacterial colonization compared with routine oral hygiene alone (Table 3).

Post hoc Tukey HSD analysis demonstrated significantly lower bacterial counts in both the Amflor and Phosflur groups compared with the control group ($P < 0.001$). However, no statistically significant difference was observed between the two fluoride mouthwash groups for either microorganism ($P > 0.05$), indicating comparable antimicrobial efficacy (Table 4).

Effect size analysis demonstrated very large treatment effects for both fluoride mouthwash groups, whereas only

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small-to-moderate changes were observed in the control group. Amflor produced the largest reduction in Streptococcus mutans, while Phosflur demonstrated a marginally greater reduction in Lactobacillus acidophilus counts; however, these differences were not statistically significant (Table 4).

IV. DISCUSSIONS

The present study evaluated the comparative antimicrobial efficacy of two commonly prescribed fluoridated mouthwashes during fixed orthodontic treatment. Fixed orthodontic appliances create additional plaque-retentive surfaces that alter the oral microbial ecosystem and increase the risk of enamel demineralization and white spot lesion formation. Brackets, archwires, and elastomeric ligatures interfere with effective mechanical plaque removal, promoting the accumulation of acidogenic and aciduric microorganisms, particularly Streptococcus mutans and Lactobacillus acidophilus. Consequently, adjunctive preventive measures capable of reducing bacterial colonization have become an integral component of contemporary orthodontic care. The present prospective randomized clinical study was undertaken to compare the antimicrobial efficacy of two commercially available fluoridated mouthwashes—amine fluoride and sodium fluoride—in reducing cariogenic bacterial colonization around orthodontic brackets after one month of use. [1-4,16-18]

The principal finding of the present investigation was that both amine fluoride and sodium fluoride mouthwashes produced a statistically significant reduction in Streptococcus mutans and Lactobacillus acidophilus counts compared with baseline, whereas no significant reduction was observed in the control group. Furthermore, both fluoride mouthwash groups demonstrated significantly lower bacterial counts than routine oral hygiene alone after one month of intervention. Although the amine fluoride group exhibited a slightly greater reduction in Streptococcus mutans counts and the sodium fluoride group demonstrated a marginally greater reduction in Lactobacillus acidophilus, the differences between the two fluoride formulations were not statistically significant. These findings indicate that both mouthwashes are effective adjuncts to routine oral hygiene in reducing cariogenic bacterial colonization during fixed orthodontic treatment.

An important strength of the present study is the absence of statistically significant differences among the study groups at baseline. The comparable pretreatment bacterial counts confirm that the randomization process successfully produced homogeneous groups and minimizes the possibility that the observed post-intervention differences resulted from pre-existing variations in oral microbial load. This baseline comparability strengthens the internal validity of the study and supports the conclusion that the reduction in bacterial counts was attributable primarily to the intervention rather than to differences in initial oral hygiene status or microbial composition.

The significant reduction in Streptococcus mutans observed in both intervention groups is consistent with the established role of fluoride in modifying the cariogenic biofilm. Fluoride has been shown to inhibit bacterial glycolysis by suppressing the activity of enolase and proton-translocating ATPase enzymes, thereby reducing acid production and impairing bacterial metabolism. In addition to its antimicrobial action, fluoride enhances enamel remineralization by promoting fluorapatite formation and increasing resistance to acid dissolution. These complementary mechanisms explain why fluoride-containing mouthwashes remain among the most widely recommended preventive adjuncts for patients undergoing fixed orthodontic treatment. [2,5,8,28]

The present findings are in agreement with previous randomized clinical trials and systematic reviews demonstrating that adjunctive fluoride mouthwashes significantly reduce cariogenic bacterial colonization and contribute to the prevention of enamel demineralization during fixed orthodontic treatment. Although both fluoride formulations significantly reduced cariogenic bacterial colonization, no statistically significant difference was observed between the Amflor and Phosflur groups. This finding suggests that, when used twice daily as an adjunct to routine oral hygiene, both formulations provide comparable antimicrobial benefits during fixed orthodontic treatment. [6-15]

The slightly greater reduction in Streptococcus mutans observed with amine fluoride and the marginally greater reduction in Lactobacillus acidophilus observed with sodium fluoride were not statistically significant and are therefore unlikely to represent clinically meaningful differences. From a clinical perspective, the choice between these formulations may therefore depend on factors such as patient preference, cost, availability, and compliance rather than superior antimicrobial efficacy.

The antimicrobial action of fluoride is multifactorial. Fluoride reduces bacterial acid production by inhibiting key metabolic enzymes, thereby decreasing the ability of cariogenic microorganisms to survive in an acidic environment. In addition, fluoride enhances remineralization of early enamel lesions and increases enamel resistance to subsequent acid attacks. Amine fluoride possesses an additional surfactant component that promotes greater surface wettability and prolonged fluoride retention on enamel, theoretically allowing longer interaction with the dental biofilm. Conversely, sodium fluoride rapidly dissociates to release fluoride ions that become readily available for remineralization and bacterial inhibition. Although these mechanistic differences suggest potential variations in antimicrobial activity, the present study indicates that both formulations ultimately achieve a similar clinical outcome after one month of regular use. [2,8,10,28]

The reduction in Streptococcus mutans observed in this study is of particular clinical importance because this organism is considered the principal initiator of dental caries and white spot lesion formation. Orthodontic brackets create

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stagnation areas that favour early colonization by S. mutans, resulting in increased acid production adjacent to the enamel surface. A reduction in its bacterial load may therefore decrease the risk of enamel demineralization during treatment. Similarly, the significant reduction in Lactobacillus acidophilus is clinically relevant because Lactobacillus species are associated with the progression of established carious lesions. Together, the observed reductions in both microorganisms suggest that adjunctive fluoride mouthwash use may contribute to a healthier oral microbial environment during fixed appliance therapy. [4,16-20]

An important feature of the present study was the standardized clinical protocol adopted for all participants. The same bracket system was used for every patient, standardized oral hygiene instructions were provided before intervention, plaque samples were collected from the same tooth at identical time intervals, and microbiological analysis was performed using standardized culture techniques. These measures minimized methodological variability and enhanced the reliability of the observed outcomes. Furthermore, the use of random allocation reduced selection bias and improved the internal validity of the study.

Another strength of the present investigation is the quantitative microbiological assessment performed using colony-forming unit counts rather than relying solely on clinical plaque indices. While plaque index scores provide valuable information regarding oral hygiene status, direct microbiological evaluation offers a more objective measure of the antimicrobial effect of preventive interventions. The use of $\log_{10}$-transformed bacterial counts, together with appropriate parametric statistical analysis and effect size estimation, further strengthens the scientific robustness of the findings.

Despite these strengths, certain limitations should be acknowledged. The study included a relatively small sample size and was conducted at a single institution, which may limit the generalizability of the findings. The follow-up period of one month was sufficient to evaluate early microbiological changes but did not permit assessment of long-term bacterial recolonization or the actual development of white spot lesions. In addition, bacterial identification was based on conventional culture techniques that quantified selected cariogenic microorganisms rather than the overall oral microbiome. Modern molecular techniques, such as quantitative polymerase chain reaction or next-generation sequencing, may provide a more comprehensive understanding of microbial changes associated with fluoride mouthwash use during orthodontic treatment.

Future research should include multicentre randomized clinical trials with larger sample sizes and longer follow-up periods to determine whether the microbiological benefits observed in the present study translate into clinically significant reductions in enamel demineralization and white spot lesion incidence. Studies incorporating advanced microbial profiling techniques and patient-reported outcomes, including taste acceptance, ease of use, and compliance, would further improve the clinical applicability of these findings. Comparative evaluation of fluoride mouthwashes with other preventive agents, including chlorhexidine, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), hydroxyapatite-based formulations, and probiotic therapies, may also provide valuable evidence for optimizing preventive protocols during orthodontic treatment. [12-15]

Overall, the findings of the present study demonstrate that both amine fluoride and sodium fluoride mouthwashes are effective adjuncts to routine oral hygiene for reducing cariogenic bacterial colonization around orthodontic brackets. Although neither formulation demonstrated clear superiority over the other, both significantly reduced Streptococcus mutans and Lactobacillus acidophilus counts compared with routine oral hygiene alone. These findings support the routine prescription of fluoridated mouthwashes as part of comprehensive preventive care for patients undergoing fixed orthodontic treatment.

V. CONCLUSION

Both amine fluoride and sodium fluoride mouthwashes significantly reduced cariogenic bacterial colonization around orthodontic brackets and may be recommended as adjuncts to routine oral hygiene during fixed orthodontic treatment.

Within the limitations of this study, both amine fluoride and sodium fluoride mouthwashes demonstrated comparable antimicrobial efficacy when used as adjuncts to routine oral hygiene during fixed orthodontic treatment.

➢ *Conflict of Interest*
The authors declare no conflict of interest.

➢ *Funding*
No external funding was received for this study.

➢ *Data Availability*
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Table 1 Normality Assessment of Log10-Transformed Colony-Forming Unit (CFU) Counts Using the Shapiro–Wilk Test

Group	Variable	W	p value	Interpretation
AMFLOR	Streptococcus (pre)	>0.90	>0.05	Normal
AMFLOR	Streptococcus (post)	>0.90	>0.05	Normal
AMFLOR	Lactobacillus (pre)	>0.90	>0.05	Normal
AMFLOR	Lactobacillus (post)	>0.90	>0.05	Normal
Control	All variables	>0.90	>0.05	Normal

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Phosflur	All variables	>0.90	>0.05	Normal

Table 2 Within-Group Comparison of Streptococcus Mutans and Lactobacillus Acidophilus Counts (log10 CFU)

Group	Microorganism	Pre (Mean ± SD)	Post (Mean ± SD)	t value	p value
AMFLOR	Streptococcus	7.431 ± 0.126	6.868 ± 0.174	12.22	<0.001*
AMFLOR	Lactobacillus	7.656 ± 0.133	7.153 ± 0.207	6.81	<0.001*
Control	Streptococcus	7.424 ± 0.128	7.536 ± 0.209	−1.45	0.182
Control	Lactobacillus	7.611 ± 0.146	7.684 ± 0.101	−1.37	0.205
Phosflur	Streptococcus	7.470 ± 0.082	6.908 ± 0.198	9.91	<0.001*
Phosflur	Lactobacillus	7.719 ± 0.131	7.098 ± 0.143	8.95	<0.001*

Table 3 Between-Group Comparisons and Post Hoc Tukey HSD Analysis at Baseline and After One Month (log10 CFU)

Microorganism	Interval / Comparison	F / Mean difference	95% CI	P value
Streptococcus	Pre-intervention (ANOVA)	F = 0.483	—	0.622
Streptococcus	Post-intervention (ANOVA)	F = 37.09	—	<0.001*
Lactobacillus	Pre-intervention (ANOVA)	F = 1.576	—	0.225
Lactobacillus	Post-intervention (ANOVA)	F = 42.64	—	<0.001*
Streptococcus	Amflor vs Control	0.668	0.452 to 0.883	<0.001*
Streptococcus	Amflor vs Phosflur	0.040	−0.175 to 0.256	0.890
Streptococcus	Control vs Phosflur	−0.627	−0.843 to −0.412	<0.001*
Lactobacillus	Amflor vs Control	0.531	0.357 to 0.705	<0.001*
Lactobacillus	Amflor vs Phosflur	−0.055	−0.228 to 0.119	0.718
Lactobacillus	Control vs Phosflur	−0.586	−0.760 to −0.412	<0.001*

*Statistically significant at P < 0.05. ANOVA, analysis of variance; HSD, honestly significant difference; CI, confidence interval.

Table 4 Effect Size for Within-Group Changes in Log10 CFU Counts

Group	Microorganism	t value	Cohen's d (dz)	Magnitude
AMFLOR	Streptococcus	12.22	3.87	Very large
AMFLOR	Lactobacillus	6.81	2.15	Very large
Control	Streptococcus	−1.45	0.46	Small–moderate
Control	Lactobacillus	−1.37	0.43	Small–moderate
Phosflur	Streptococcus	9.91	3.14	Very large
Phosflur	Lactobacillus	8.95	2.83	Very large

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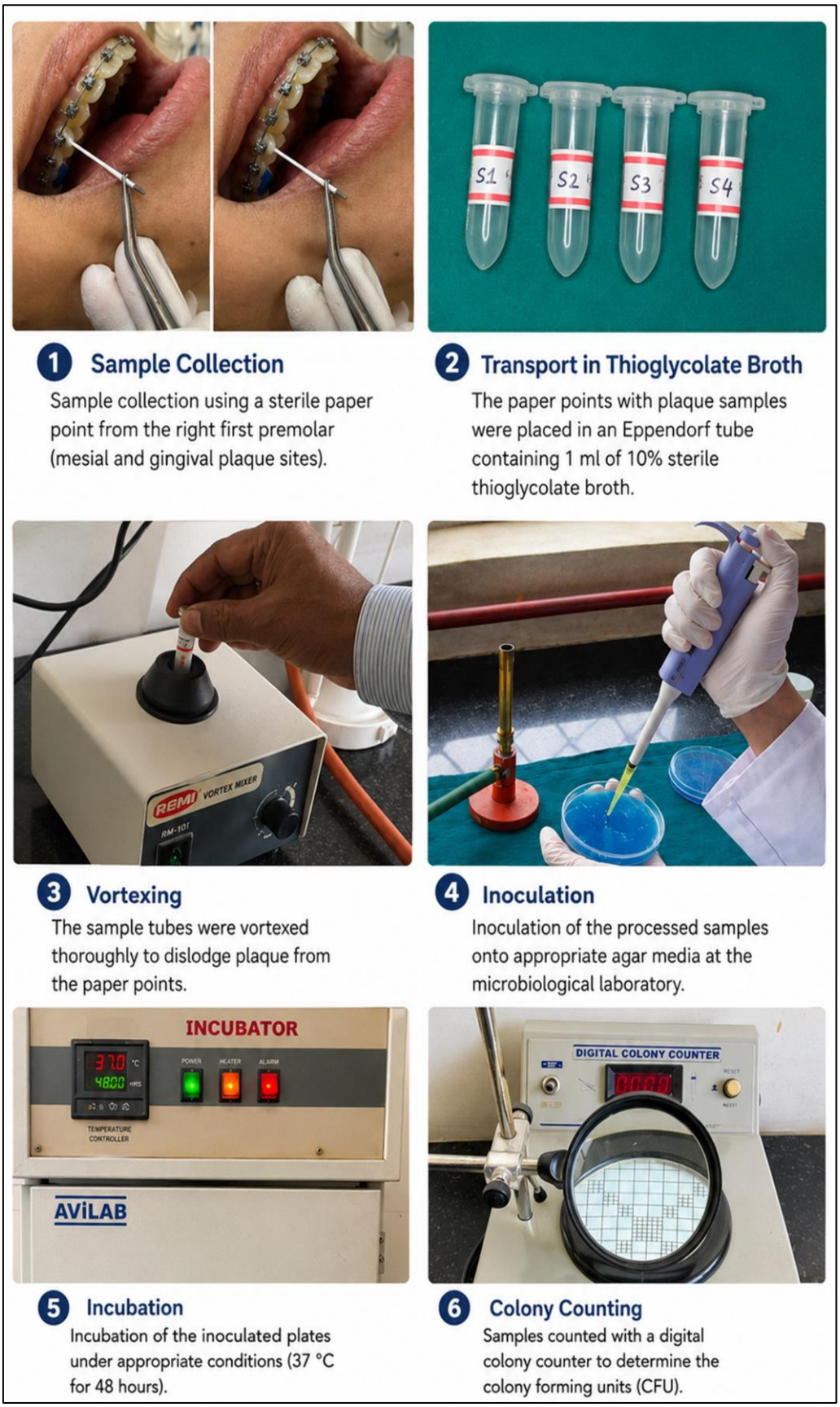


Fig 1 Workflow of plaque sample collection and microbiological processing. (1) Plaque sample collection from the mesial and gingival sites of the right first premolar using a sterile paper point; (2) transport of the paper points containing plaque samples in an Eppendorf tube containing 1 mL of 10% sterile thioglycollate broth; (3) vortexing of the sample tubes to dislodge plaque from the paper points; (4) inoculation of the processed samples onto appropriate agar media; (5) incubation of the inoculated plates at 37°C for 48 hours; and (6) enumeration of colonies using a digital colony counter to determine colony-forming units (CFU).

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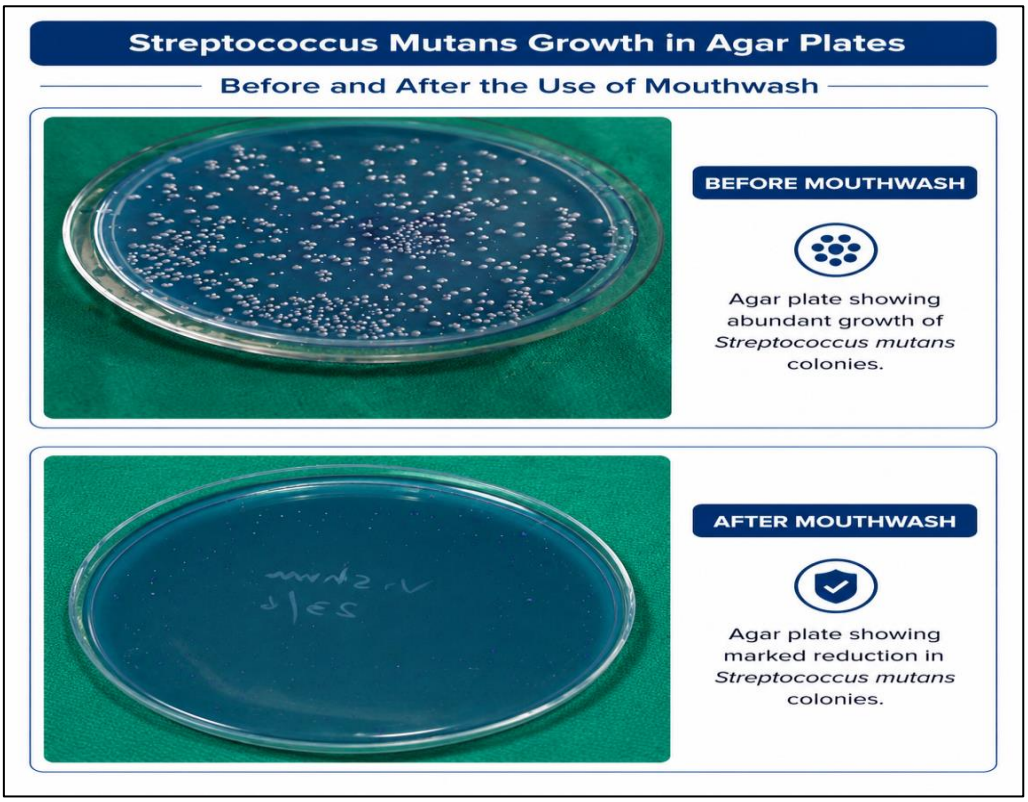


Fig 2 Representative Mitis Salivarius Bacitracin agar plates showing Streptococcus mutans growth before and after the use of mouthwash. The upper panel shows abundant colony growth before mouthwash use, whereas the lower panel shows a marked reduction in colonies after mouthwash use.

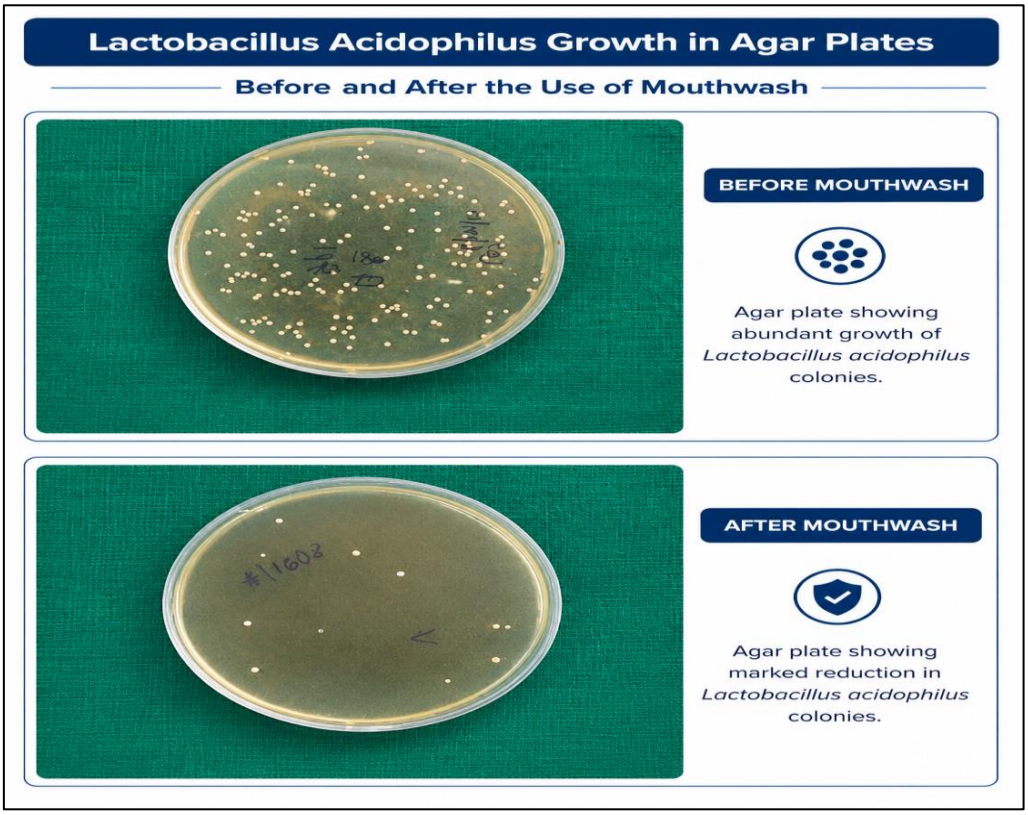


Fig 3 Representative de Man, Rogosa and Sharpe agar plates showing Lactobacillus acidophilus growth before and after the use of mouthwash. The upper panel shows abundant colony growth before mouthwash use, whereas the lower panel shows a marked reduction in colonies after mouthwash use.

- Abbreviations: CFU, colony-forming units.

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