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A Comparative Assessment of the Antibacterial Efficacy of Alum Mouthwash Versus 0.2% Chlorhexidine Mouthwash in Reducing Streptococcus Mutans on Essix Orthodontic Retainers: An In-Vitro Study.

Dr. Vedant B. Gambhire[1], Dr. Nitin Gulve[2], Dr. Amit Nehete[3], Dr. Rashmi Patil[4], Dr. Prerna Badgujar[1], Dr. Ruchita Tayade[1].

1. Student, Department of Orthodontics and Dentofacial Orthopedics, M.G.V.'s K.B.H Dental College and Hospital, Nashik
2. Head of the Department, Professor, Department of Orthodontics and Dentofacial Orthopedics, M.G.V.'s K.B.H Dental College and Hospital, Nashik
3. Professor, Department of Orthodontics and Dentofacial Orthopedics, M.G.V.'s K.B.H Dental College and Hospital, Nashik
4. Assistant Professor, Department of Orthodontics and Dentofacial Orthopedics, M.G.V.'s K.B.H Dental College and Hospital, Nashik

Running title

Antibacterial efficacy of Alum mouthwash on essix orthodontic retainer

Corresponding Author: Dr. Vedant Bhausaheb Gambhire, Student, Department of Orthodontics and Dentofacial Orthopedics, M.G.V.'s K.B.H Dental College and Hospital, Nashik

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KEYWORDS

Streptococcus mutans, Essix retainer, Alum mouthwash

ABSTRACT:

Introduction: An Essix orthodontic retainer is the most popular form of post-orthodontic retention. Orthodontists generally advise their patients to wear their retainers long-term to prevent relapse. Chlorhexidine is well-known, widely used antimicrobial agent for plaque and gingival health control, which may be suitable for cleaning removable orthodontic appliances in order to reduce the plaque buildup on their surfaces. Potash Alum is characterized by its odorless nature, affordability, and low toxicity when used in small amounts. Research indicates that Potash Alum is notably effective in diminishing plaque and reducing salivary levels of oral streptococci.

Objectives: The objective is to evaluate the antibacterial efficacy of Alum mouthwash and 0.2% Chlorhexidine mouthwash in decreasing the presence of *Streptococcus mutans* on Essix orthodontic retainers.

Methods: A total 15 pressure - formed retainers were fabricated from Essix retainer sheets on duplicates of maxillary cast and then cut down each single retainer into three parts. Essix retainer were randomly selected and sterilized by immersion in 1% sodium hypochlorite for 30 min. After sterilization the Essix retainer were removed with sterile forceps and rinsed thrice in sterile distilled water. The Essix retainer were then placed in the plaque solution consisting of 15 ml of sterile sucrose, 30 ml of Brain Heart Infusion broth and 5 ml of *Streptococcus mutans* and were incubated aerobically at 37º C. Forty-five Essix retainer pieces were randomly and equally divided into 3 groups (15 Essix retainer pieces in each group). The biofilms were cultivated for 72 hours in plaque solution. After cultivation of *S.mutans* biofilm, the Essix retainer were removed and colony forming unit baseline was calculated, which were then subjected and dipped in to the mouthwashes for 10 minutes as follows; GROUP 1: (Control Group) Essix retainer kept without cleaning kept in

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Artificial saliva GROUP 2: Soaked essix retainer into the Alum mouthwash. GROUP 3: Soaked essix retainer into the 0.2% Chlorhexidine mouthwash. For Groups 2 and 3, the essix retainer were dipped into Alum and 0.2% chlorhexidine mouthwash for 10 min and diluting immediately in a 5% phosphate buffer solution and then the phosphate buffer solution was subjected for colony forming count on petri dish and after 72 hours, *streptococcus mutans* colonies were counted manually. Quantitative assessment was carried out by comparing the number of viable colonies of *Streptococcus mutans*. A One-way ANOVA followed by Tukey,s post hoc test was used to compare the data between the experimental and control groups. ($p < 0.05$).

Results Group 1 (No cleaning) showed a post-treatment mean count of 10.40 ± 2.69, Group 2 (Alum Mouthwash) had a mean count of 6.13 ± 1.60, and Group 3 (Chlorhexidine Mouthwash) demonstrated the lowest mean count of 2.67 ± 2.41. When compared to untreated controls the antimicrobial efficacy of Alum and 0.2% Chlorhexidine Di gluconate mouthwashes was found to be statistically significant ($p = <0.001$). Chlorohexidine mouthwash showing the greatest effectiveness in reducing *Streptococcus mutans.*

Conclusions: A 0.2% Chlorhexidine mouthwash should be use for its antibacterial properties as a rinsing solution to inhibit the growth of *Streptococcus mutans* on Essix orthodontic retainer. While alum mouthwash is not as effective as the 0.2% Chlorhexidine mouthwash in providing antibacterial action, it can still serve as an alternative mouthrinse to prevent the proliferation of *Streptococcus mutans* on Essix Orthodontic retainer.

1. Introduction

An Essix orthodontic retainer is the most popular form of post-orthodontic retention. Its ease of fabrication and good aesthetics means that it is cost effective and has increased patient compliance. Orthodontists generally advise their patients to wear their retainers long-term to prevent relapse[1].

However, these devices have disadvantages such as loosening over time, discoloration, fracture and crack formation, limitation of the washing and buffering effects of saliva on teeth. In addition, the thermoplastic retainer in the mouth affects the oral flora in favor of the cariogenic bacteria *Streptococcus mutans* and *Lactobacillus*[2].

High level of *Streptococcus mutans* were detected in plaque from saliva and the surfaces of carious teeth as well as sound teeth. A significant virulence characteristic of the bacterium is its capacity to develop biofilm, commonly referred to as dental plaque, on the surfaces of teeth. In addition, this organism also produces glucosyl-transferases, multiple glucan-biding proteins, protein antigen c, and collagen-binding protein, surface proteins that coordinate to produce dental plaque, thus inducing dental caries[3].

Chlorhexidine is well-known, widely used antimicrobial agent for plaque and gingival health control, which may be suitable for cleaning removable orthodontic appliances in order to reduce the plaque buildup on their surfaces. Chlorhexidine Di gluconate has been recognized as a Gold Standard for chemical plaque control since the 1970s and is particularly effective in cleaning orthodontic retainers, thereby reducing plaque accumulation on Essix retainer. This cationic compound exhibits strong efficacy against bacterial biofilms by disrupting their external cellular structures and compromising the integrity of bacterial cytoplasmic membranes[3].

Potash Alum ($KAl(SO4)2·12H2O$) is a naturally occurring substance recognized for its antibacterial and antifungal characteristics. This compound has been utilized since ancient times by civilizations such as the Egyptians, Indians, and Chinese. In the Indian subcontinent, it is commonly referred to as Phitkary and is a staple in many households[4]. Potash Alum is characterized by its odorless nature, affordability, and low toxicity when used in small amounts. Its safety has led to approval by the United States Food and Drug Administration as a food additive. Research indicates

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that Potash Alum is notably effective in diminishing plaque and reducing salivary levels of oral streptococci [5].

There have been different In-vitro studies on different cleaning methods to remove *Streptococcus mutans* from Essix orthodontic retainer to different extents. These studies have compared the efficiency of using chemical method for reducing *Streptococcus mutans* from Essix orthodontic retainer.

There is no published data on effectiveness of Alum mouthwash which has antibacterial properties to reduce *Streptococcus mutans* from Essix orthodontic retainer.

Therefore, the objective is to evaluate the antibacterial efficacy of Alum mouthwash and 0.2% Chlorhexidine mouthwash in decreasing the presence of *Streptococcus mutans* on Essix orthodontic retainers.

2. Objectives

I) To evaluate the colony forming unit of *Streptococcus mutans* on Essix orthodontic retainer without cleaning.

II) To evaluate the colony forming unit of *Streptococcus mutans* on Essix orthodontic retainer after soaking it into the Alum mouthwash for 10 minutes.

III) To evaluate the colony forming unit of *Streptococcus mutans* on Essix orthodontic retainer after soaking it into the 0.2% Chlorhexidine mouthwash for 10 minutes.

IV) To compare the colony forming unit of *Streptococcus mutans* on Essix orthodontic retainer after soaking in into the Alum mouthwash and 0.2% Chlorhexidine mouthwash.

3. Methods

This in vitro study was carried out in the Department of Orthodontics and Dentofacial Orthopedics & Department of Microbiology and Pathology.

The sample comprises of 45 Essix retainer pieces.

A total 15 pressure - formed retainers were fabricated from Essix retainer sheets on duplicates of maxillary cast and then cut down each single retainer into three parts (figure 1). Essix retainer were selected and sterilized by immersion in 1% sodium hypochlorite for 30 min. After sterilization the Essix retainer were removed with sterile forceps and rinsed thrice in sterile distilled water. The Essix retainer were then placed in the plaque solution consisting of 15 ml of sterile sucrose, 30 ml of Brain Heart Infusion broth and 5 ml of *Streptococcus mutans* and were incubated aerobically at 37° C. Forty-five Essix retainer pieces were randomly and equally divided into 3 groups (15 Essix retainer pieces in each group). The biofilms were cultivated for 72 hours in plaque solution. After cultivation of *S.mutans* biofilm, the Essix retainer were removed and colony forming unit baseline was calculated, which were then subjected and dipped in to the mouthwashes for 10 minutes as follows (figure 2) -

GROUP 1: (Control Group) Essix retainer kept without cleaning kept in Artificial saliva

GROUP 2: Soaked essix retainer into the Alum mouthwash.

GROUP 3: Soaked essix retainer into the 0.2% Chlorhexidine mouthwash.

For Groups 2 and 3, the essix retainer were dipped into Alum and 0.2% chlorhexidine mouthwash for 10 min and diluting immediately in a 5% phosphate buffer solution and then the phosphate buffer solution was subjected for colony forming count on petri dish and after 72 hours, *streptococcus mutans* colonies were counted manually.

Fig.1 Essix retainer

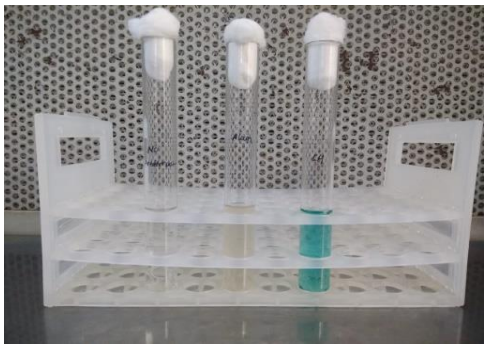

Fig.2 Essix retainer dipped into mouthwashes

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Statistical analysis: -

The data are tabulated in Microsoft excel and analysed with SPSS V.24 software. The continuous variables are presented with mean and standard deviation. The categorical variables are presented with frequency and percentage. Paired t test and One way ANOVA are used for the statistical analysis. The p value ≤0.05 is considered statistically significant.

4. Results

Table 1, depicts the baseline count of colony forming unit count of all samples. Table 2 depicts the colony forming unit count of all samples after treatment. Table 3 and figure 3, shows the comparison of *Streptococcus mutans* counts before and after treatment in Group 1 (No cleaning) it demonstrates a significant reduction in bacterial counts. Before treatment, the mean count was 304.20 ± 22.40, which decreased to 10.40 ± 2.69 after kept in artificial saliva. The removal ability of *Streptococcus mutans* in this group was calculated as 96.58%. The results were statistically significant, with a p-value of <0.001.

Table 4 and figure 4, shows the comparison of *Streptococcus mutans* counts before and after treatment in Group 2 (Alum Mouthwash) also shows a significant reduction in bacterial counts. The mean count before treatment was 300.73 ± 22.16, which reduced to 6.13 ± 1.60 after treatment. The removal ability in this group was 97.96%. The results were statistically significant, with a p-value of <0.001.

Table 5 and figure 5, shows the comparison of *Streptococcus mutans* counts before and after treatment in Group 3 (Chlorhexidine Mouthwash) also reveals a significant reduction in bacterial counts among the groups. The mean count before treatment was 307.73 ± 16.96, which dropped to 2.67 ± 2.41 after treatment. The removal ability of *Streptococcus mutans* in this group was the highest at 99.13%. The results were statistically significant, with a p-value of <0.001.

Table 6 and figure 6, shows the post-treatment comparison of *Streptococcus mutans* counts between the three groups highlights the differences in efficacy among the interventions. The p-value of <0.001 indicates a statistically significant difference in the post-treatment bacterial counts between the groups, with Group 3 showing the greatest effectiveness in reducing *Streptococcus mutans*.

Table 1. Baseline count of Colony forming unit of *streptococcus mutans.*

Sr. No	Group 1(control)	Group 2(Alum Mouthwash)	Group3 (cholorohexidine mouthwash)
1	359 X 10^5	322X10^5	284X10^5
2	270 X 10^5	337X10^5	294X10^5
3	355X10^5	339X10^5	308X10^5
4	288X10^5	289X10^5	288X10^5
5	322X10^5	280X10^5	290X10^5
6	287X10^5	275X10^5	309X10^5
7	290X10^5	288X10^5	317X10^5
8	293X10^5	295X10^5	320X10^5
9	344X10^5	298X10^5	333X10^5
10	287X10^5	302X10^5	321X10^5
11	309X10^5	333X10^5	309X10^5
12	300X10^5	307X10^5	299X10^5
13	290X10^5	289X10^5	302X10^5
14	280X10^5	277X10^5	298X10^5
15	289X10^5	280X10^5	344X 10^5

Table 2- Colony forming unit count of *streptococcus mutans* after treatment

Sr. No	Group 1(control)	Group 2(Alum Mouthwash)	Group 3 (cholorohexidine mouthwash)
1	09 X10^5	08 X10^5	09 X10^5
2	13 X10^5	07 X10^5	03 X10^5
3	12 X10^5	06 X10^5	02 X10^5
4	11 X10^5	03 X10^5	02 X10^5
5	09 X10^5	07 X10^5	02 X10^5
6	08 X10^5	08 X10^5	01 X10^5

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7	07 X10^5	06 X10^5	01 X10^5
8	07 X10^5	06 X10^5	-
9	06 X10^5	07 X10^5	03 X10^5
10	09 X10^5	08 X10^5	03 X10^5
11	12 X10^5	05X10^5	07 X10^5
12	14 X10^5	05 X10^5	02 X10^5
13	14 X10^5	03 X10^5	03 X10^5
14	13 X10^5	07 X10^5	02 X10^5
15	12 X10^5	03 X10^5	-

Table 3-Comparison of *Streptococcus mutans* counts before re and after kept in artificial saliva Group 1 (No cleaning)

Group	Time	Mean	SD	Removing ability	P value
Group 1(No cleaning)	Before	304.20	22.40	96.58%	<0.001
	After	10.40	2.69		

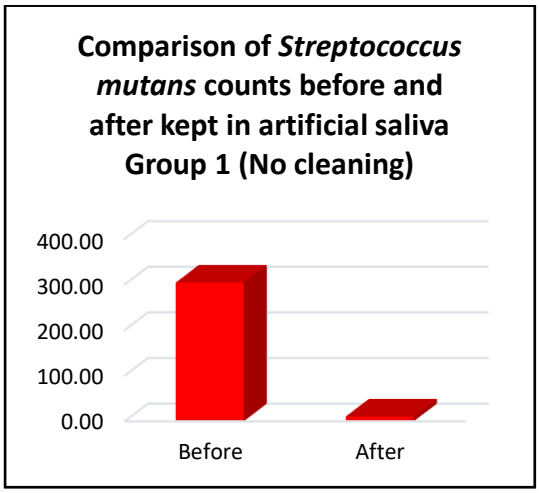

Fig.3, comparison of *Streptococcus mutans* count before and after kept in artificial saliva group 1(no cleaning).

Table 4-Comparison of *Streptococcus mutans* counts before and after treatment in Group 2 (Alum Mouthwash)

Group	Time	Mean	SD	Removing ability	P value
Group 2 (Alum Mouthwash)	Before	300.73	22.16	97.96%	<0.001
	After	6.13	1.60		

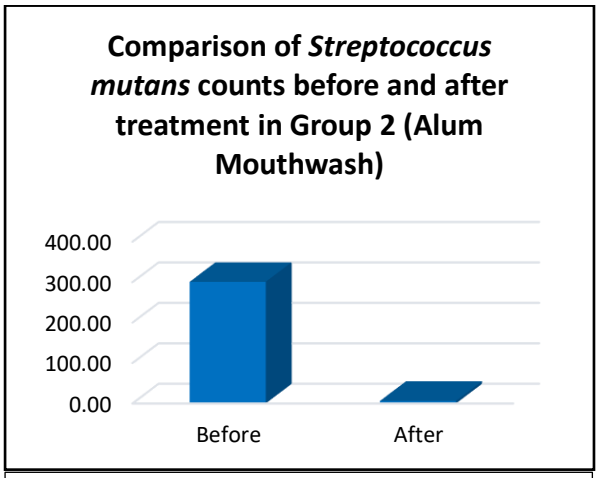

Fig.4, comparison of *Streptococcus mutans* count before and after treatment in group 2(Alum mouthwash).

Table 5-Comparison of *Streptococcus mutans* counts before and after treatment in Group 3 (Chlorhexidine Mouthwash)

Group	Time	Mean	SD	Removing ability	P value
Group 3 (chlorhexi-ne mouth-wash)	Before	307.73	16.96	99.13%	<0.001
	After	2.67	2.41		

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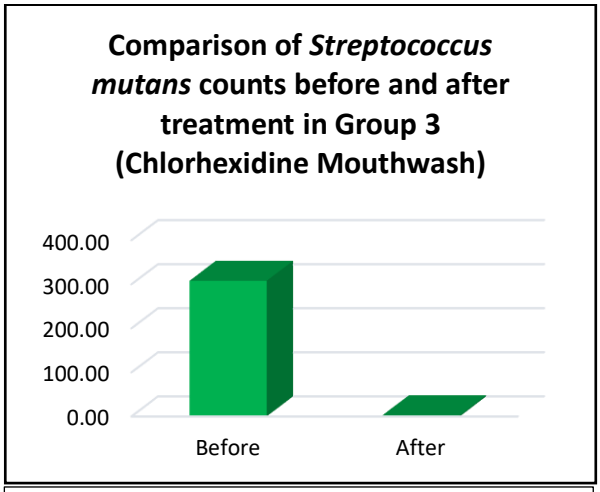

Fig.5, Comparison of *Streptococcus mutans* counts before and after treatment in Group 3 (Chlorhexidine Mouthwash).

Table 6-Comparison of *Streptococcus mutans* counts after treatment between the groups

Group	Mean	SD	P value
Group 1 (No cleaning)	10.40	2.69	<0.001
Group 2 (Alum Mouthwash)	6.13	1.60	
Group 3 (Chlorhexidine Mouthwash)	2.67	2.41	

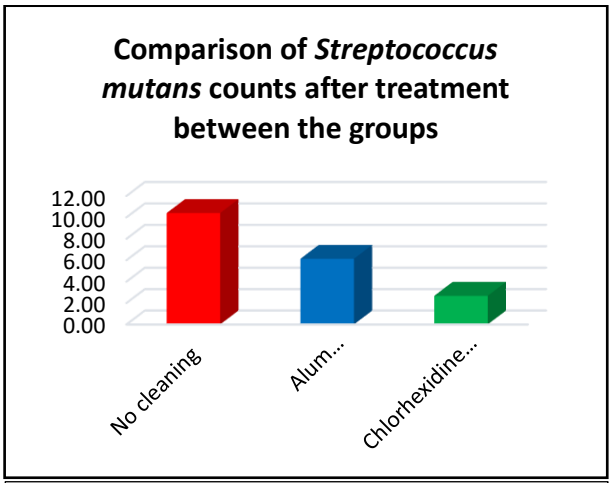

Fig.6, Comparison of *Streptococcus mutans* counts after treatment between the groups.

5. Discussion

Maintaining diligent oral hygiene is considerably challenged by the presence of orthodontic devices, which promote the accumulation and stagnation of plaque on the orthodontic removable appliances. In pursuit of a new cleaning agent that could serve as an alternative to chlorhexidine, the present study examined and evaluated the antibacterial effectiveness of Alum and Chlorhexidine for cleaning essix retainer.

The current investigation sought to evaluate and contrast the antimicrobial effectiveness of Alum and 0.2% Chlorhexidine mouthwashes against *Streptococcus mutans* biofilm on Essix orthodontic retainers. The findings indicated that the application of Chlorhexidine mouthwash on the Essix retainer led to a relatively more decrease in colony-forming unit count compared to treatment with Alum mouthwash.

In the present investigation, a sample size of 45 was categorized into three groups, each containing 15 samples. Essix retainers were immersed in a plaque solution composed of 15 ml of sterile sucrose, 30 ml of Brain Heart Infusion (BHI) broth, and 5 ml of *Streptococcus mutans*. This mixture was incubated aerobically at 37 degrees Celsius for 72 hours prior to the division into the three groups.

The control group, designated as Group 1, exhibited the highest count of colony-forming units, as it was the group immersed in artificial saliva. This was followed by Group 2 (Alum mouthwash), while Group 3 (Chlorhexidine mouthwash) recorded the lowest count. A significant difference was noted when comparing the two mouthwashes, Group 2 and Group 3.

Al-Huwaizi RF et al[5] (2013) investigated the effects of different concentrations of Alum, from 50 to 10,000 PPM, on the inhibition zone, viability counts, and adherence ability of *Mutans streptococci* in vitro. The results revealed that lower concentrations of Alum enhanced the adherence of *Mutans streptococci* to tooth surfaces. Conversely, higher concentrations of 5,000 and 10,000 PPM produced effects akin to those observed with 0.1% and 0.2% chlorhexidine, leading to reduced adherence.

Vanishree BK et al.[4] (2021) evaluated the impact of alum mouthwash on plaque control in children aged 9 to 12 years and the findings indicated that both alum-based mouthwash and herbal mouthwash were effective in

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enhancing plaque inhibition. The findings of our study indicate that the reduction is substantial, and the application of Alum cleaning agent as an alternative to Chlorhexidine exhibits a modest yet clear inhibitory effect against the microorganisms examined. Given that this research has demonstrated a significant decrease in colony forming unit count with the use of alum, additional steps should be considered for its implementation in cleaning of essix retainer. In the human oral cavity, a diverse array of microorganisms coexists, engaging in interspecies interactions that contribute to increased resistance and virulence against antimicrobial agents. Nevertheless, this study focused exclusively on Streptococcus mutans, a bacterium associated with dental caries, which may not accurately reflect the broader ecological context. Additionally, the research did not establish the ideal frequency for chemical cleaning of retainers, nor did it investigate the long-term effects on the physical properties of the materials, including paint adhesion or aging.

6. Conclusion

A 0.2% Chlorhexidine mouthwash should be use for its antibacterial properties as a rinsing solution to inhibit the growth of Streptococcus mutans on Essix orthodontic retainer. While alum mouthwash is not as effective as the 0.2% Chlorhexidine mouthwash in providing antibacterial action, it can still serve as an alternative mouthrinse to prevent the proliferation

of *Streptococcus mutans* on Essix Orthodontic retainer.

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