

CLINICAL AND MICROBIOLOGICAL EFFECTIVENESS OF CHLORHEXIDINE AND SODIUM HEXAMETAPHOSPHATE MOUTH RINSES ON DENTAL PLAQUE IN CHILDREN

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ABSTRACT

Introduction: The use of a mouthwash augments mechanical removal of plaque by brushing and flossing and helps maintain oral health through its antiplaque and antibacterial chemical properties.

Aim: to evaluate and compare clinically and microbiologically the effectiveness of chlorhexidine and sodium hexametaphosphate mouth rinses on dental plaque in children.

Materials and methods: This randomized clinical trial involved 40 normal apparently healthy and cooperative children aged 8 to 10 years and chosen from the Outpatient Dental Clinic of Pediatric dentistry Department, Faculty of Dentistry, Suez Canal University. Children were randomly divided into 2 groups as follows: Group **I**: included (20) children who rinsed with Chlorhexidine (0.2%) and Group **II**: included (20) children who rinsed with Sodium Hexametaphosphate (7%). Clinical evaluation was performed by measuring gingival and plaque indices scores at all evaluation periods. Microbiological evaluation was performed by direct examination of bacterial cultures.

Results: Sodium hexametaphosphate 7% mouth rinse showed a superior effect compared to chlorhexidine 0.2% mouth rinse in terms of improvement in the clinical scores and decreasing the microbial counting.

Conclusion: Sodium hexametaphosphate 7% mouth rinse is a promising candidate of mouthwashes.

KEYWORDS: Chlorhexidine; Dental plaque; *Lactobacillus*; Sodium Hexametaphosphate; *Streptococcus mutans*.

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INTRODUCTION

Dental plaque is a biofilm that is naturally formed on surfaces of teeth and other parts of the oral cavity. It is considered as the main etiological factor for oral diseases; specially dental caries and periodontal diseases⁽¹⁾.

Mechanical plaque control is the mainstay for prevention of oral diseases, but it requires patient cooperation and motivation; therefore, chemical plaque control is used for achieving the desired results.⁽²⁾ Chemotherapeutic agents may be used to reduce, eliminate or change the quality of microbial pathogens. In addition, they alter the host response through local or systemic delivery of appropriate agents.⁽³⁾ Chlorhexidine is one of the most frequently used compounds. It is a broad spectrum antiseptic with strong antimicrobial effects on both Gram-positive as well as Gram-negative bacteria and some viruses.⁽⁴⁾

Chlorhexidine remains in the oral cavity several hours after application that makes it a useful agent for prevention of plaque regrowth and bacterial colonization.⁽⁵⁾

On the other hand, chlorhexidine has some disadvantages; it irritates the oral mucosa and this is not dosage dependent. Tooth discoloration, sensitivity changes, taste perturbation, unilateral, or bilateral parotid swelling and tongue pain may occur as side effects leading to search for new formulations.⁽⁶⁾

The addition of inorganic polyphosphate salts (including sodium hexametaphosphate) to fluoridated products has been shown to be effective in increasing their effectiveness against caries.⁽⁷⁾ Sodium hexametaphosphate (SHMP) is able to inhibit the formation of dental calculus,⁽⁸⁾ has antimicrobial action⁽⁹⁾ and also prevents the formation of extrinsic stains. SHMP has greater possibility of binding to the enamel surface and protecting it from acid dissolution.⁽¹⁰⁾

The aim of this study was to compare and evaluate the effect of chlorhexidine and sodium hexametaphosphate mouth rinses on dental plaque in children.

MATERIALS AND METHODS

This study was a randomized clinical trial involving 40 normal apparently healthy and cooperative children aged from 8-10 years from both sexes from those attending Outpatient Clinic of Pediatric and Preventive Dentistry and Dental Public Health Department, Faculty of Dentistry, Suez Canal University. Adequate diagnosis and treatments planning were performed. Each selected child was subjected to full mouth treatments if needed until we had all the selected patients with no caries.

Inclusion criteria:

- Either caries free children or previously treated children.
- Age from 8-10 of both sexes.
- Apparently healthy children.
- No systemic diseases, children took antibiotic, steroid therapy or any anti-inflammatory drugs for the last 3 weeks.
- Child with full eruption of upper left permanent first molar, upper left permanent lateral incisor, upper right primary first molar, lower right permanent first molar, lower right permanent lateral incisor and lower left primary first molar.

Exclusion criteria:

- Children with orthodontic appliances or with intra oral prosthesis.
- Un cooperative children or children who cannot attend the follow up visits.
- Children had a history of fluoride treatment in the past 2 weeks.
- Allergy to any dental products or their ingredients.

- Patients with missing upper left permanent first molar or upper left permanent lateral incisor or upper right primary first molar or lower right permanent first molar or lower right permanent lateral incisor or lower left primary first molar.

Informed consent, both written and verbal, was obtained from both the parent and the children before enrolling in the study. Each child was instructed to brush his or her teeth with a toothbrush wetted with water using Stillman's brushing technique. Tooth brushing technique and mouth rinsing method were demonstrated for every child. Children were instructed to brush and rinse twice daily in the morning and evening for two weeks. They were instructed to rinse with 15 ml of the solution for 1 min followed by expectoration of the residual mouth rinse and they were asked not to eat or drink anything for 30 min after using the mouthwash for collection of plaque samples. During the treatment period there was not any other oral hygiene measure.^(11, 12)

Children were randomly divided into 2 groups as follows:

- Group I: included (20) children who rinsed with Chlorhexidine (0.2%).
- Group II: included (20) children who rinsed with Sodium Hexametaphosphate (7%).

Preparation of chlorhexidine (0.2%) mouth wash

Mouth wash containing chlorhexidine was formulated by the following formula.⁽¹³⁾ A calculated amount of surfactant was heated and transferred to the main container. Sorbitol was diluted with distilled water and added to the main container. In other containers, Sodium benzoate and Sodium saccharine were dissolved, filtered and added to the main container. Chlorhexidine was diluted with water, filtered and added to the main container. Finally flavouring agent and colour were dissolved separately, filtered and added to the main container.

The total volume was adjusted using distilled water. The final product was analyzed and filled in suitable bottles.

Preparation of Sodium Hexametaphosphate (7%) mouth wash:

Mouth wash containing Sodium hexametaphosphate was formulated by the following formula⁽¹³⁾ A calculated amount of surfactant was heated and transferred to the main container. Sorbitol was diluted with distilled water and added to the main container. In other containers, Sodium benzoate and Sodium saccharine were dissolved, filtered and added to the main container. Sodium hexametaphosphate was diluted with water, filtered and added to the main container. Finally, flavouring agent and colour were dissolved separately, filtered and added to the main container. The total volume was adjusted using distilled water. The final product was analyzed and filled in suitable bottles.

Methods of evaluation:

All children were evaluated clinically and microbiologically at (the base line) a day before using mouth washes, then at 7th and 15th day after mouth rinsing..

A- Clinical evaluation:

Clinical evaluation by measuring gingival and plaque indices scores was done as follows:

Gingival index:¹⁴

Gingival index (Loe H. and Silness J.1963) was used to evaluate the gingival condition and record qualitative changes in the gingiva. The examination was done by blunt probe and partially erupted teeth was excluded. The selected teeth were upper left permanent first molar, upper left permanent lateral incisor, upper right primary first molar, lower right primary first molar. The selected surfaces were buccal, lingual, mesial and distal.

Turesky, Gilmore, Glickman modification of the Quigley Hein Plaque Index (Turesky et al. Modified Quigley Hein Plaque Index):⁽¹⁵⁾

A disclosing agent was used for plaque detection. Children were instructed to rinse with the disclosing agent. The selected teeth were the same used above in the gingival index. Plaque was assessed on the labial, mesial, distal and lingual surfaces of the teeth after using a disclosing agent.

B- Microbiological analysis

I- Collection of specimens:

Sterile cotton swab fixed on a wooden stick was streaked across the entire labial tooth surface of the lower anteriors (incisors) from distal to mesial in a horizontal direction while exerting pressure on the swab getting as close as possible to the gingival margin without touching it (supragingival plaque). All the samples were aseptically processed and tested for the isolation, identification and colony counts of *Streptococcus mutans* and *Lactobacillus* species.

II-*Streptococcus mutans* isolation and identification:

Streptococcus mutans are facultative anaerobic Gram-positive coccus-shaped bacteria and their optimal growth is at 37°C. On mitis salivarius agar, the colonies of *Streptococcus mutans* are small, raised, irregularly margined and adherent.^(16,17)

Preparation of mitis salivarius bacitracin (MSB) agar:

Mitis salivarius agar was used to selectively enumerate *Streptococcus mutans* in swabs from dental plaque.⁽¹⁸⁾ The MSB medium was prepared according to the instructions of the manufacturer.

III-Lactobacilli isolation and identification:

Lactobacilli are long, slender, non-spore forming, Gram-positive rods that are generally facultative anaerobic, most of which grow well

with reduced oxygen tension and increased CO₂. *Lactobacilli* grow on a variety of media including Rogosa agar where they appear as white, usually mucoid colonies.⁽¹⁹⁾

Preparation of Rogosa agar:

Rogosa agar was used to selectively enumerate *lactobacilli* in the swabs from dental plaque.⁽²⁰⁾ The medium was prepared according to the instructions of the manufacturer.

IV-Processing of the Specimens:

1. Swabs were placed into tubes containing 5 ml tryptone soya broth. Samples were vortexed for 30 seconds to mix them thoroughly.
2. For quantitative bacterial assessment, sterile disposable calibrated loops 1/100 and 1/1000 ml were used to transfer and streak swab specimens into freshly prepared MSB and Rogosa agar plates.
3. (MSB) agar plates were incubated anaerobically at 37°C for 48-72 hours.
4. Rogosa agar plates were incubated anaerobically in anaerobic jar using anaerobic gas pack and plates were incubated at 37°C for 48-72 hours.
5. After incubation, a colony counter with magnifying glass was used to count the number of colonies and they were expressed as the number of colony forming units per ml (CFU/ml) of sample. By multiplying the actual colony count by 10² or 10³ (according to the used calibrated loop) quantification of the number of colonies was done.⁽²¹⁾

Statistics:

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, student t- test, Paired t-test by SPSS V20.

$$\text{Mean} = \frac{\sum x}{n}$$

Where Σ = sum & n = number of observations.

$$1. \text{ Sta } \sqrt{\frac{\Sigma |x - \bar{x}|^2}{n-1}} \text{ on [SD]} : \\ SD = \sqrt{\frac{\Sigma |x - \bar{x}|^2}{n-1}}$$

Student t-test [Unpaired]:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{SE_1^2 + SE_2^2}}$$

Where:

\bar{X}_1 = Mean of the first group .

\bar{X}_2 = Mean of the second group .

SE_1 = Standard error of the first group.

SE_2 = Standard error of the second group.

Unpaired Student T-test was used to compare between two groups in quantitative data.

Paired t-test

$$t = \frac{\bar{X}_d}{\sqrt{SE_d}}$$

Where:

\bar{X}_d = Mean's difference between pre and post .

SE_d = Standard error of the difference between pre and post.

Unpaired Student T-test was used to compare between related sample

P-value > 0.05 Non significant

P-value ≤ 0.05 Significant

P-value < 0.01 Highly Significant

RESULTS

Clinical results

Evaluation of clinical parameters (gingival and plaque indices scores) was done for all the treated groups at baseline, 7 day and 15 day according to the gingival index system and Turesky et al Modified Quigley Hein Plaque Index system respectively.

Gingival index results:

Group I and group II showed a decrease in the mean value of gingival index form baseline to 7 days, from 7 days to 15 days and from baseline to 15 days. This decrease was highly statistically significant difference** as shown in table (1).

In between groups, there was a highly statistical significant decrease** in the mean of gingival index when comparing between group II and group I at all evaluation periods (after 7 days and 15 days) as shown in table (1).

1.2 Turesky et al. Modified Quigley Hein Plaque Index results:

Group I and Group II showed a decrease in the mean value of plaque index form baseline to 7 days, from 7 days to 15 days and from baseline to 15 days. This decrease was highly statistically significant difference** as shown in table (2) and figures (1, 2).

In between groups, at the 7th day there was a statistical significant decrease* in the mean value of plaque index in group II when compared with its corresponding value in group I.

At the 15th day there was highly statistically significant decrease** in the mean value of plaque index in group II when compared with its corresponding value in group I as shown in table (2).

2- Microbiological results

2.1 Streptococcus mutans count:

The mean value of *S. mutans* count in both group I and group II showed a highly statistically significant decrease** from 7 days to 15 days and from baseline to 15 days as shown in table (3) and figures (3,4).

In between groups, there was a highly statistical significant decrease** in the mean of *S. mutans* count when comparing between group II and group I at all evaluation periods (after 7 days and 15 days) as shown in table (3).

TABLE (1): Comparison between mean values of gingival index in group I and group II measured at different times of evaluation.

Gingival index		Groups		T-Test	
		group I	group II	T	P-value
Baseline	Range	0.41 - 0.81	0.04 - 0.79	-1.572	0.124
	Mean ±SD	0.09± 0.57	0.17± 0.50		
After 7 Days	Range	0.17 - 0.71	0.13 - 0.54	-4.145	0.001**
	Mean ±SD	0.12 ± 0.47	0.12± 0.31		
After 15 Days	Range	0.13 - 0.58	0.04 - 0.38	-4.435	0.001**
	Mean ±SD	0.11± 0.31	0.11 ± 0.15		
B-7D	Differences	Mean ±SD	0.10 ± 0.05	0.19 ± 0.11	
	Paired Test	P-value	0.001**	0.001**	
B-15D	Differences	Mean ±SD	0.26 ± 0.06	0.34 ± 0.13	
	Paired Test	P-value	0.001**	0.001**	
7-15D	Differences	Mean ±SD	0.16 ± 0.06	0.15 ± 0.05	
	Paired Test	P-value	0.001**	0.001**	

The group I = Chlorhexidine group - The group II = Sodium Hexametaphosphate group

*P-value > 0.05 Non significant * P-value ≤ 0.05 Significant **P-value < 0.01 Highly Significant*

TABLE (2): Comparison between mean values of Turesky et al Modified Quigley Hein Plaque Index in the group I and group II measured at different times of evaluation.

Plaque index			Groups		T-Test	
			Group I	Group II	T	P-value
Baseline		Range	0.63 - 1.5	0.5 - 1.54	-0.671	0.506
		Mean ±SD	0.22 ± 0.96	0.29 ± 0.91		
After 7 Days		Range	0.42 - 1.17	0.25 - 1.13	-2.307	0.027*
		Mean ±SD	0.18 ± 0.73	0.22 ± 0.58		
After 15 Days		Range	0.13 - 0.96	0.04 - 0.79	-2.709	0.010**
		Mean ±SD	0.19 ± 0.52	0.18 ± 0.35		
B-7D	Differences	Mean ±SD	0.23 ± 0.12	0.32 ± 0.13		
	Paired Test	P-value	0.001**	0.001**		
B-15D	Differences	Mean ±SD	0.44 ± 0.16	0.55 ± 0.20		
	Paired Test	P-value	0.001**	0.001**		
7-15D	Differences	Mean ±SD	0.21 ± 0.06	0.23 ± 0.10		
	Paired Test	P-value	0.001**	0.001**		

The group I = Chlorhexidine group, group I = Sodium Hexametaphosphate group

*P-value > 0.05 Non significant * P-value ≤ 0.05 Significant **P-value < 0.01 Highly Significant*

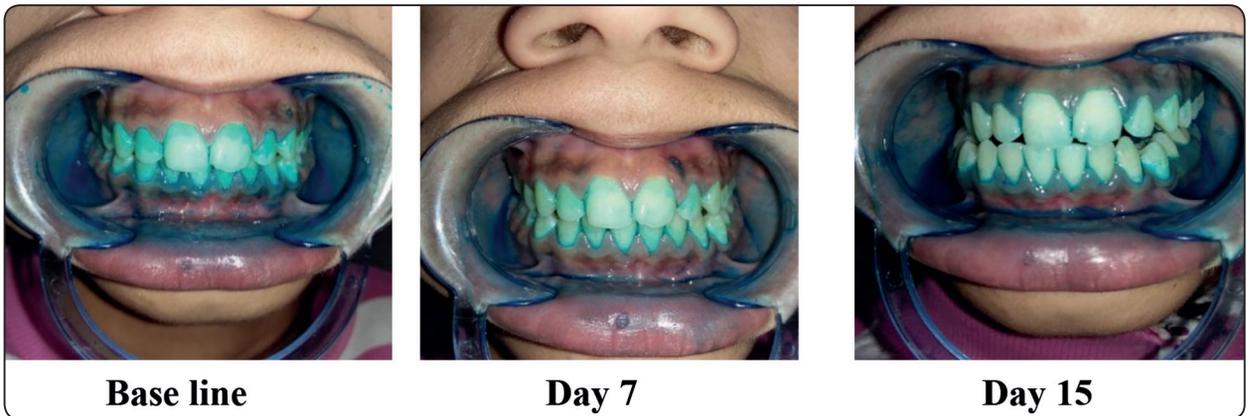


Fig. (1) Showing patient 10 years old showing staining of microbial biofilm in dental plaque with disclosing agent at base line, day 7 and day 15 (group I).



Fig. (2) Showing patient 10 years old showing staining of microbial biofilm in dental plaque with disclosing agent at base line, day 7 and day 15 (group II).

TABLE (3): Comparison between *S. mutans* count in group I and group II measured at different times of evaluation.

S. Mutants count			Groups						T-Test	
			Group I			group II			t	P-value
Baseline	Range	4.6	-	5.08	4.48	-	5.18	0.635	0.529	
	Mean ±SD	4.90	±	0.15	4.94	±	0.20			
After 7 Days	Range	4	-	5	3.3	-	4.7	-4.596	0.001**	
	Mean ±SD	4.75	±	0.30	4.11	±	0.54			
After 15 Days	Range	3.7	-	4.95	2.7	-	4.48	-6.374	0.001**	
	Mean ±SD	4.41	±	0.49	3.36	±	0.54			
B-7D	Differences	Mean ±SD	0.14	±	0.37	0.82	±	0.48		
	Paired Test	P-value	0.097			0.001**				
B-15D	Differences	Mean ±SD	0.49	±	0.53	1.57	±	0.53		
	Paired Test	P-value	0.001**			0.001**				
7-15D	Differences	Mean ±SD	0.35	±	0.43	0.75	±	0.34		
	Paired Test	P-value	0.002**			0.001**				

The group I = Chlorhexidine group, group II = Sodium Hexametaphosphate group
 P-value > 0.05 Non significant * P-value ≤ 0.05 Significant



Fig. (3) showing *Streptococcus mutans* colony growth on Mitis salivarius bacitracin agar (MSB) at base line, day 7 and day 15 (group I).

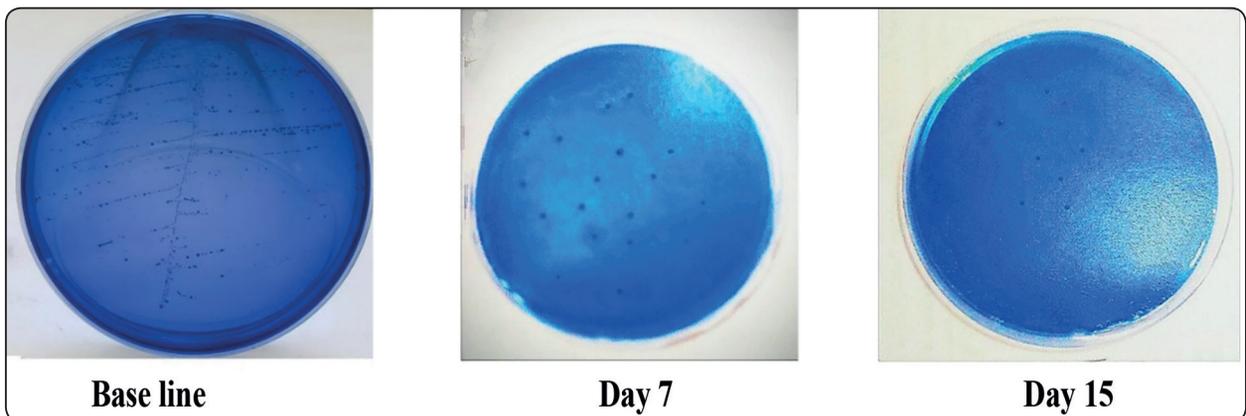


Fig. (4) showing *Streptococcus* growth colony on Mitis salivarius bacitracin agar (MSB) at base line, day 7 and day 15 (group II).

2.2 *Lactobacillus* count:

The mean value of *Lactobacillus* count in both group I and group II showed a highly statistically significant decrease** from 7 days to 15 days and from baseline to 15 days as shown in table (4) and figures (5, 6).

In between groups, there was no statistical significant difference in the mean value of *Lactobacillus* count when comparing between group II and group I at all evaluation periods (after 7 days and 15 days) as shown in table (4).

TABLE (4): Comparison between Lactobacillus count in group I and group II measured at different times of evaluation.

Lactobacillus count		Groups						T-Test	
		Group I			Group II			t	P-value
Baseline	Range	3.7	-	4.9	4	-	5	1.579	0.123
	Mean ±SD	4.44	±	0.46	4.64	±	0.32		
After 7 Days	Range	3.3	-	4.88	2.99	-	4.7	-1.901	0.065
	Mean ±SD	4.25	±	0.51	3.90	±	0.63		
After 15 Days	Range	2.7	-	3.7	2.7	-	3.48	-0.951	0.348
	Mean ±SD	3.07	±	0.4	2.97	±	0.27		
B-7D	Differences	Mean ±SD	0.20	±	0.20	0.74	±	0.36	
	Paired Test	P-value	0.001**			0.001**			
B-15D	Differences	Mean ±SD	1.37	±	0.58	1.67	±	0.35	
	Paired Test	P-value	0.001**			0.001**			
7-15D	Differences	Mean ±SD	1.17	±	0.61	0.93	±	0.65	
	Paired Test	P-value	0.001**			0.001**			

The group I = Chlorhexidine group, group II = Sodium Hexametaphosphate group

P-value > 0.05 Non significant

* P-value ≤ 0.05 Significant

**P-value < 0.01 Highly Significant

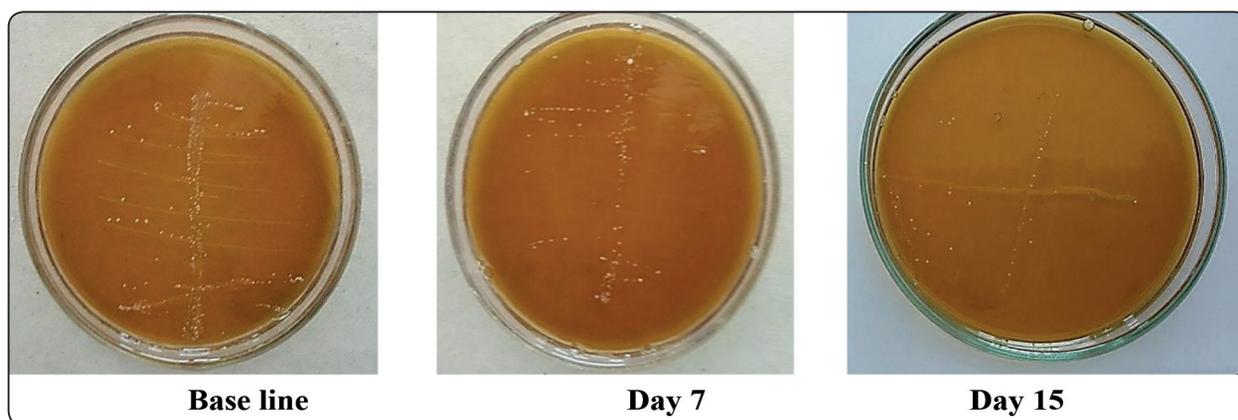


Fig. (5) Showing Lactobacillus colony growth on Rogosa agar at base line, day 7 and day 15 (group I).

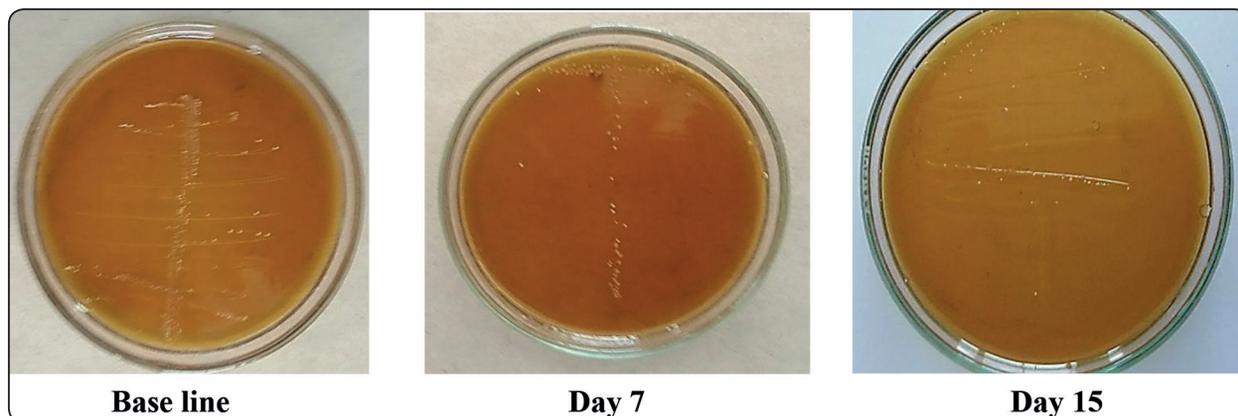


Fig. (6) showing *Lactobacillus* colony growth on Rogosa agar at base line, day 7 and day 15 (group II).

DISCUSSION

Dental plaque is a deposition that comprised of numerous living species of microorganisms embedded in an extracellular matrix. Formation of dental plaque is a progressive and dynamic process, which may result in the establishment of caries and gingivitis. *Streptococcus mutans* and *lactobacilli* are the chief components of dental plaque.⁽²²⁾

In order to prevent mouth diseases, plaque control is achieved by regular mechanical means and chemical agents, which act specifically against those microorganisms.⁽²³⁾

Chlorhexidine was used as a mouthwash because it is considered the gold standard anti plaque agent against which efficacy of other anti-plaque and anti-gingivitis agents is measured. In addition, it gives immediate bactericidal and prolonged bacteriostatic actions due to adsorption onto the pellicle-coated enamel surface.^(24, 25)

In this study, Sodium Hexametaphosphate (SHMP) was used because it is a non-combustible material with no significant environmental effects. It has a low oral toxicity and may cause minor irritation to skin, eyes and respiratory tract.⁽²⁶⁾

Clinical results of this study demonstrated that gingival and Turesky et al Modified Quigley Hein Plaque indices scores among treated children

with SHMP mouth rinse had statistical significant improvement compared to the group treated with chlorhexidine mouth rinse at all evaluation periods (7 days and 15 days).

The effect of chlorhexidine on plaque and its property of slow release have made it as the panacea in the adjunctive treatment to gingivitis. Chlorhexidine being cationic prevents pellicle formation and is bacteriostatic at lower concentrations and bactericidal at higher concentrations.⁽²⁷⁾

Bellamy et al.⁽²⁸⁾ stated that (SHMP) when added to a stabilized stannous fluoride dentifrice provides the additional benefits of prevention of calculus formation, stain removal and reduction of stain formation and plaque formation.

Microbiological results of this study revealed that the mean value of *S. mutans* count showed a statistical significant decrease when comparing between II and group I at all evaluation periods (7 days and 15 days). This may be due to the potent antimicrobial activity of SHMP.

Antibacterial effect of SHMP 7% mouthwash against *S. mutans* and *lactobacillus* were better than that produced by chlorhexidine 0.2% mouthwash.

Many studies showed that (SHMP) inhibits the growth of *streptococci mutans* resulting in a decrease of plaque formation and dental caries.⁽²⁹⁾

On other hands, SHMP does not generally cause any adverse effect on the body when used locally and orally within the range of minimum inhibitory concentrations (MICs) determined for various bacteria.⁽³⁰⁾

In addition, Hacchou et al.⁽³¹⁾ found that SHMP can stimulate bone formation. Thus, SHMP seems to be a promising substance for treatment of periodontal diseases and promoting bone regeneration.

These results were in agreement with Murphy et al.⁽³²⁾, Da Camara et al.⁽³³⁾ who proved that SHMP is characterized by potent, and various biological properties including efficient anti-microbial and anti-biofilm actions. SHMP probably have multiple mechanisms of antibacterial action.

The antimicrobial effect of SHMP can be explained by a mechanism suggested by Maier et al.⁽³⁴⁾ as SHMP may inhibit the microorganisms by forming potentially inhibitory compounds (e.g., superoxide) or disrupting intracellular metabolism or protein folding.⁽³⁵⁾

In addition, Shibata et al.⁽³⁶⁾ and Knabel et al.⁽³⁷⁾ reported that the antibacterial effect of SHMP against Gram-positive bacteria, including *Streptococci mutans*, is related to its ability to chelate divalent cations resulting in inhibition of cell division and loss of cell wall integrity. SHMP induces direct lysis of *Staphylococcus aureus* (*S. aureus*) by chelation of structurally essential metal ions in their membranes.^(38, 39)

Also, the antimicrobial action of SHMP is related to its capacity to increase the permeability of the bacteria and glucose transport when it links to the Mg⁺ present in the outer membrane.⁽⁴⁰⁾

These results agreed with an in vitro study carried out by Post et al.⁽⁴¹⁾ in which the influence of SHMP on selected bacteria was assessed. The results showed that most Gram-positive bacteria

were prevented from growing on medium containing 0.1% SHMP and Gram-negative bacteria were capable of growing in higher concentrations even up to 10% SHMP.

In addition, another study in 2008 carried out by Obritsch et al.⁽⁴²⁾ in which the sensitivity of Gram-negative (*Escherichia coli* [*E. coli*] and *Salmonella Typhimurium*) and Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Lactobacillus plantarum* and *Listeria monocytogenes*) spoilage and pathogenic bacteria to different members of long-chain polyphosphates including SHMP was tested. The results showed that Gram-positive bacteria are more sensitive to polyphosphates than are Gram-negative bacteria, possibly because of the differences in cell wall structure.

CONCLUSIONS

1- SHMP mouthwash at a concentration of 7% had superior antibacterial effects.

2-SHMP7% mouthwash improved the clinical parameters than chlorhexidine 0.2% mouthwash (gingival index scores and Turesky et al Modified Quigley Hein Plaque Index scores).

3-SHMP7% mouthwash decreased the microbial count of *Streptococcus mutans* and *Lactobacillus* species than chlorhexidine 0.2% mouthwash.

Limitations of the study

1- Selection of the patients with the inclusion criteria was difficult and took a long time.

- Chemical preparation of mouthwashes was difficult especially SHMP mouth wash.
- Media used for isolation, identification and colony counts of *Streptococcus mutans* and *Lactobacillus* species were not available.
- The disclosing agent was not present in Egypt and was brought from Germany.

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