The Influence of Chlorhexidine Gluconate and Povidone Iodine on The Minimum Inhibitory Concentration and Quantitative of *Streptococcus mutans*

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Received 7 November 2024; 1st revision 15 August 2025; Accepted 29 August 2025; Published online 29 August 2025

Keywords:

Dental caries, Streptococcus mutans; minimum inhibitory concentration,chlorhexidine, povidone iodine

ABSTRACT

Background: *An imbalance in the normal flora of the oral cavity can cause dental caries. The prevalence of caries in primary teeth in Indonesia which experiences caries is 90.2%, which shows that the prevalence of caries is still quite high. One way to prevent bacterial activity is using mouthwash. Chlorhexidine gluconate and Povidone Iodine are antibacterial agents that are effective in inhibiting bacterial growth, through bacteriostatic and bactericidal actions. This study aims to determine whether there is an inhibitory effect of chlorhexidine gluconate and povidone iodine on the growth of Streptococcus mutans (quantity).*

Method: *This study used the broth microdilution method by treating Streptococcus mutans using 0.1 % chlorhexidine gluconate and 1% povidone iodine. The effect of inhibitory power was measured using the broth microdilution technique and the total plate count technique in the form of Colony Forming Units (CFU). Statistical calculations use the Shapiro-Wilk normality test, Levene's homogeneity test and Tukey's Post Hoc test to determine differences between treatments.*

Result: *The results of the research showed that the treatment group in the form of 0.1% chlorhexidine gluconate and 1% povidone iodine had a very significant effect on the growth of bacterial colonies, which was very significant for the growth of Streptococcus mutans bacterial colonies.*

Conclusion: *Chlorhexidine gluconate 0.1% and povidone iodine 1% were effective in reducing the growth of Streptococcus mutans bacteria.*

doi: http://dx.doi.org/10.30659/odj.12.183-189
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How to Cite: Apriani *et all*. The Influence of Chlorhexidine Gluconate and Povidone Iodine on The Minimum Inhibitory Concentration and Quantitative of Streptococcus mutans. Odonto: Dental Journal, v.12, n.2, p.183-189 August 2025.

INTRODUCTION

The human body is a host for trillions of microbes, and the oral cavity is one of the largest sources of microbes, there are around 700 to 1000 species of microbes found in the human oral cavity.[1] Microorganisms in the oral cavity include bacteria, yeast, viruses, mycoplasma, and protozoa which form a heterogeneous ecological system in the oral cavity known as the oral microbiota.[2] The interaction of various oral microorganisms helps the human body fight the invasion of unwanted stimuli from the outside. Imbalance of microbial flora can cause oral diseases such as dental caries, periodontitis, oral mucosal diseases and systemic diseases.[3] Cariogenic streptococcus can produce water-insoluble glucans from sucrose, and in addition can facilitate adhesion (attachment of organisms to the tooth surface, serving as a source of nutrients and matrix for plaque development). The relationship between sucrose and dental caries is very complex and cannot be explained simply by calculating the total amount of sugar consumed. There are carbohydrates other than sucrose, for example glucose and fructose, which are also cariogenic, but less cariogenic than sucrose. Polyol carbohydrates, 'sugar alcohol' for example xylitol, with low cariogenicity have been produced and used as sugar substitutes such as chewing gum and baby food.[4]

The dominant bacteria that causes caries is *Streptococcus mutans. Streptococcus mutans* is a gram-positive bacteria in the oral cavity, has a coccus shape, is an encapsulated bacterium and produces acid which is considered an extracellular glucan polymer.[5] The virulence factor of Streptococcus mutans is capable of producing organic acids (acidogenic), the ability to survive in low pH and synthesize glucosyltransferase (GTF) which is able to convert sucrose into glucose.[6] *Streptococcus mutans* produces Lipo Teichoic Acid (TCA) which is able to bind directly to the enamel surface and cause bacterial colonization.[7]

Chlorhexidine gluconate is a bisbiguanide compound, works quickly and has low toxicity and its a broad spectrum antimicrobial agent that can disrupt the cell membrane and cell wall permeability of gram-positive and gram-negative bacteria. Chlorhexidine gluconate was biocompatible and is used orally as an antiseptic mouthwash as protection against germs and is able to prevent bacterial biofilm and plaque accumulation.[8] When compared with other bacteria involved in plaque formation, *Streptococcus mutans* is more easily killed using chlorhexidine gluconate.[9]

Povidone iodine was a chemical compound that combines polyvinylpyrrolidone with iodine compounds. Its has broad spectrum antibacterial activity with activity against bacteria including strains, fungi that are resistant to antibiotics, antiseptics and protozoa.[10] The antimicrobial action of povidone iodine involves the formation of permanent pores in the cell membranes of microorganisms.[11] Povidone iodine can kill bacteria by inhibiting bacterial enzyme metabolism which will interfere with bacterial multiplication and cause the bacteria to become weak. Povidone iodine can reduce the severity of gingivitis, mucositis and can be used for caries prevention because it has long-term preferential and antimicrobial effects against Streptococcus mutans.[12]

The use of topical antimicrobial agents in the oral cavity, such as chlorhexidine gluconate and povidone iodine, has long been a cornerstone strategy for the prevention and control of periodontal diseases. Chlorhexidine gluconate at 0.1% was selected for its effectiveness in reducing plaque accumulation and gingival inflammation during short-term use, with minimal risk of irritation, whereas the 0.2% concentration was employed for its faster bactericidal action and often recommended in the postoperative phase of periodontal surgery.[13,14] Povidone iodine at 0.5% was commonly used for daily rinsing or pre-procedural mouthwash due to its ability to reduce bacterial and viral load without causing significant burning sensations, while the 1%

concentration was more frequently applied for periodontal pocket irrigation and oral surgical procedures to ensure optimal antiseptic penetration.[15,16] The selection of these concentrations reflects a balance between antimicrobial efficacy, oral tissue safety, and patient comfort, thereby enabling clinical applications tailored to individual needs.Based on the background, research was conducted regarding the use of chlorhexidine gluconate and povidone iodine mouthwash as a supporting ingredient for dental and oral health care. The aim of the research is to determine the inhibitory effect of chlorhexidine gluconate and povidone iodine on *Streptococcus mutans*.

RESEARCH METHOD

This research is an in vitro laboratory experimental study using the spread plate technique. The research was carried out using the broth microdilution method with 4 treatment groups, namely chlorhexidine gluconate 0.1%, chlorhexidine gluconate 0.2%, povidone iodine 0.5%, povidone iodine 1%. Apart from that, a control group was used in the form of distilled water, growth control and clindamycin. The media used in this research were Brain-heart Infusion Broth (BHIB) and Mueller Hinton Agar (MHA).

Measurement of Inhibition Levels

The test for determining the MIC value (Minimum Inhibitory Level) is determined as the smallest concentration that can inhibit bacteria as indicated by the clarity of the growth medium in the tube after incubation at a temperature of ± 370 C for ± 24 hours.

Quantity Measurement of *Streptococcus mutans*

The number of bacterial colonies was counted using the Total Plate Count (TPC) technique in the form of Colony Forming Units (CFU).

In this research, a normality test will be carried out first using the Shapiro-Wilk test and followed by a homogeneity test using the Levene test, then to determine the results of the data hypothesis test using the paired T-test.

RESULTS

Based on the research results, the number of colonies of *Streptococcus mutans* bacteria was calculated before and after adding 0.1% chlorhexidine gluconate, 1.2% clindamycin (positive control), and distilled water (negative control group). Tabel 1

Table 1. The number of bacterial colonies before and after adding 0.1% chlorhexidine gluconate

No	Chlorhexidine gluconate CFU		Clindamycin 1,2% (K+) CFU		*Aquadest*t (K-) CFU	
	Before	After	Before	After	Before	After
1.	143	45	141	0	138	133
2.	145	43	147	0	131	136
3.	151	39	138	0	145	141
4.	135	37	153	0	142	147
5.	147	40	157	0	148	142
6.	155	38	139	0	136	140

7.	142	29	144	0	129	126
8.	145	32	140	0	138	132
9.	147	33	139	0	132	140
Mean	145.6	37.3	144.2	0	137.7	137.4
Decrease (%)	74.3		100		0.00	

The results showed that the average bacterial colony before and after adding 1.2% clindamycin, distilled water, and 0.1% chlorhexidine gluconate decreased. Chlorhexidine gluconate 0.1% (treatment) showed a significant decrease, before treatment the number of colonies was 145, while after treatment it was only 37, which was a 74.3 % decrease from the initial number. With clindamycin 1.2% (positive control) there was a large decrease in the average number of bacteria, before treatment the number of colonies was 144, while after treatment there were no remaining colonies, which shows a decrease in the number of colonies of 100%. In distilled water (negative control) the number of bacterial colonies was previously 138, whereas after being added the remaining number of colonies was 137, which shows 0% decrease (Table 1).

Table 2. The number of bacterial colonies before and after adding 1% povidone iodine

No	Povidone Iodine CFU		Clindamycin 1,2% (K+) CFU		*Aquadest*t (K-) CFU	
	Before	After	Before	After	Before	After
1.	155	11	166	0	161	157
2.	148	10	171	0	165	162
3.	168	16	153	0	157	164
4.	136	13	162	0	166	159
5.	157	11	158	0	147	152
6.	144	13	169	0	149	155
7.	161	10	149	0	131	143
8.	152	12	151	0	140	151
9.	158	10	176	0	152	147
Mean	153.2	11.8	161.7	0	152	154.4
Decrease (%)	92.3		100		0.01	

The average data on bacterial colonies before and after treatment shows that the number of bacterial colonies with 1% povidone iodine treatment, before the treatment was given, was 153 and after being given the number was 12 or there was a decrease in the number of bacteria by 92.3% from before the treatment was given. In the treatment with 1.2% clindamycin as a positive control, the number of bacterial colonies before treatment was 162 and after treatment there were 0 or there was a decrease in the number of bacterial colonies by 100% from before treatment. In the treatment with distilled water as a negative control, there were 152 bacterial colonies before treatment and 154 after treatment, or there was no decrease in the number of bacterial colonies (0%) from before treatment (Table 2).

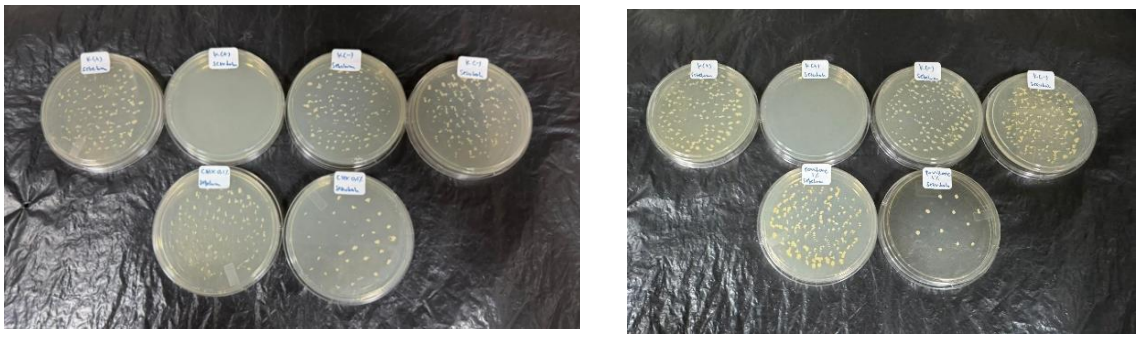
Table 3. Statistical Test Results (Paired T-test)

Group	Mean	Sd	Value t	Value p

control +	144.222	6.797	63.659	0.000
control -	0.222	5.608	0.119	0.908*
Chlorhexidine gluconate	108.22	7.242	4.832	0.000

control +	141.444	0.593	44.233	<0.001
control -	-2.444	7.248	-1.012	0.171*
Povidone iodine	161.667	9.539	50.842	<0.001

The results of data hypothesis testing using the paired T-test, it can be concluded that the treatment group in the form of 1% povidone iodine and the positive control in the form of 1.2% clindamycin had an influence on the growth of *Streptococcus mutans* bacterial colonies with a p value or significance <0.05. When compared based on standard deviation or SD, the treatment group in the form of 1% povidone iodine was more effective than the positive control in the form of 1.2% clindamycin (Table 3).



(a) **(b)**

Figure 1. *Streptococcus mutans* bacterial colonies before and after being given 0.1% chlorhexidine gluconate solution, 1.2% clyndamicin and aquadest (a). *Streptococcus mutans* bacterial colonies before and after being given 1% povidone iodine solution, 1.2% clyndamicin and aquadest (b).

The image shows that the average number of bacterial colonies before and after adding 1.2% clindamycin, 1% povidone iodine and 0.1% chlorhexidine gluconate decreased, while the addition of distilled water did not result in a decrease in the number of S*treptococcus mutans* colonies (Figure 1).

DISCUSSION

This research was conducted with the aim of comparing the differences in the number of colonies before and after adding 0.1% chlorhexidine gluconate and 1% povidone iodine. The positive control group had an average number of bacterial colonies of 0.000 CFU/ml. Clindamycin works by preventing the formation of peptides thereby inhibiting protein synthesis, which is carried out by attaching to the 50S subunit of bacterial ribosomes.[17] The negative control group had a mean of 0.212 CFU/ml. This happens because the compound from distilled water has neutral properties and does not have antibacterial activity.[18]

Chlorhexidine's ability to absorb and adhere to negatively charged surfaces is key to its success as an antimicrobial agent. Chlorhexidine molecules have the ability to adsorb on anionic substrates such as

hydroxyapatite, pellicle, salivary glycoproteins, and mucous membranes, resulting in a direct bactericidal effect. When released slowly from the pellicle-coated enamel surface, chlorhexidine exerts a sustained bacteriostatic effect in the body (a process called substantivity).[19] Substantivity is the ability of mouthwash to adhere and remain on the surface of the oral cavity and the substantivity of chlorhexidine is 12- 18 hours.[20,21] Chlorhexidine can be used as an additional drug in the treatment of gingivitis for 4 to 6 weeks and can significantly reduce plaque buildup. The optimal dose for using chlorhexidine mouthwash is 20mg twice a day with a gargle time of 30-60 seconds.[21]

The treatment group used in the form of povidone iodine in this study, povidone iodine is considered to have the broadest spectrum of antimicrobial action when compared with other common antiseptics such as chlorhexidine, octenidine, polyhexanide which show effectiveness against gram-positive and gram-negative bacteria, bacterial spores, fungi, protozoa and some viruses. Persistence effects have also been tested in a study evaluating poviodone iodine 1% as a pre-procedural antibacterial agent in individuals with varying levels of oral hygiene. The reduction in the concentration of microorganisms was found to be maintained for at least 4 hours.[22] The research results illustrate that compared to other antiseptics such as chlorhexidine, PVP-I or povidone iodine has stronger antibacterial power and better virucidal activity. After the release of free iodine from polyvinylpyrrolidone, povidone iodine works to quickly penetrate into microorganisms, disrupt protein structures through oxidation of nucleic acid structures, and ultimately kill microbes.[23]

The research results was in line with the theory that povidone iodine is an antiseptic with the broadest antimicrobial spectrum compared to antiseptics in general, which is effective in killing gram-positive and gram-negative bacteria, bacterial spores, fungi, protozoa and viruses. In research conducted by Lestari et al, with povidone iodine as a mouthwash with concentrations of 0.2%, 0.4%, 0.8% and 1% respectively with a duration of 15 seconds and 30 seconds, it was found that the Povidone iodine is able to kill bacterial colonies such as S*treptococcus mutans*, from hundreds to tens of bacteria before treatment, using only the lowest concentration variation, namely 0.2% povidone iodine for 15 seconds.[24]

CONCLUSION

Based on the results of the research that has been carried out and the discussion, it can be concluded that Chlorhexidine gluconate 0.1% and Povidone iodone 0.1% have an optimal level of effectiveness in killing S*treptococcus mutans* bacteria.

ACKNOWLEDGEMENT

The author would like to thank the Faculty of Dentistry Maranatha Christian University for invaluable support for this research. Besides, we would like to express my appreciation to Thio Lison and Nabila Syofie AM for their assistance in this research.

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