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Antibacterial Effect of Mouthwashes against Selected Bacteria

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ABSTRACT

Today, with the development of the concept of preventive dentistry and the awareness of the society, the oral care habits of individuals have developed, especially in urban areas. The aim here is to spread protective practices and this awareness to the whole society. While oral care habits are limited to brushing teeth, the importance given to interface cleaning today has increased the importance of using dental floss interface brush, toothpick and mouthwash. Research shows how important the use of mouthwash is for oral and dental health. Initially, mouthwashes, which were used for cosmetic purposes such as removing bad breath, are now becoming an indispensable part of oral care habits due to their many benefits.In this *in vitro* study, the antibacterial effects of six different mouthwashes on *S.mutans*, *E.faecalis*, *Bacillus subtilis*, *Lactobacillus casei*, *S. aureus* were investigated using the Agar Diffusion Test. In order to examine the antibacterial effects of different mouthwashes, 7 slots of 5 mm width were opened on the agar plates. Each mouthwash was placed in these slots and one was left blank as the control group. After 24 hours, evaluations were made by measuring the areas of inhibition against the tested microorganisms. Kruskal Wallis and Mann Whitney U Test were used for statistical analysis of the results. The tested mouthwashes were found to have antibacterial effects on 5 different micro-organisms ($P < 0.05$). It was concluded that the preparation numbered six was the most effective in terms of the inhibition zone thicknesses they created. ($P < 0.05$).

Keywords: Antibacterial effect, mouthwash, Jordan, In-vitro study,S Mutans, E faecalis, Lactobacillus casei,Bacillus subtilis

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INTRODUCTION

Bacterial colonization on the tooth surface is the most important etiological agent of common oral diseases (tooth decay, gingivitis, destructive periodontal diseases). Many microorganisms, especially *S. mutans*, play a role in pit and fissure caries[1]. In order to control tooth decay and periodontal diseases and to prevent bad breath, mouthwashes can be recommended by the clinician in dental care procedures. In the last decade, the indication for use of mouthwash is often recommended "following a good mechanical cleaning to ensure biofilm control"[2].

Biocides that destroy or inhibit the growth of microorganisms on living tissues are called antiseptics. They are less toxic than disinfectants, which are similar, but differ from antiseptics due to their use on inanimate objects and surfaces[3] . Resistance to multiple substances today is a public health problem that has been observed worldwide after the appearance of antibiotics. An example that offers evolutionary signs of resistance is the bacterium *Staphylococcus aureus*. The indiscriminate use of antibiotics and the selective environmental pressure carried out by antiseptics and disinfectants has generated a survival response in microorganisms, which enables them to effectively evade the bactericidal action of some of these chemical agents (2). During the last 20 years, the indiscriminate use of these products has made bacteria endowed with multiple mechanisms (biochemical, genetic-molecular and cellular) develop inherent and acquired strategies, which allow them to effectively evade the action of these chemical compounds[4].But medical personnel have not always had a clear understanding of this phenomenon or of the modulating role that the application of a correct policy for the use of these substances has on it.An important factor to take into account when establishing a policy for the use of disinfectants and antiseptics is the standardization of quality control methods for these products according to the nature, physical state of the substances and the use for which they are intended. During the Dental Practice, both in University Dentistry clinics and in the health sector and in private practice, various antiseptics are being used.[5]However, although there is information on these, it has not been clear or explicit about the concentration have the best antibacterial effect. Therefore, the effectiveness of these must be more studied and periodically since today bacteria are becoming tolerant and / or resistant to various chemical substances to which they were previously susceptible.Supragingival plaque accumulation inevitably leads to gingivitis and periodontitis develops from localized gingivitis. The specific physiological mechanisms of the host and bacteria that induce the transition from gingivitis to periodontitis are not fully known, therefore the prevention of periodontal disease is based on the reduction of plaque accumulation. If to this we add the insufficient mechanical control of it, either due to incorrect brushing technique or due to inadequate oral hygiene habits in a large part of the population, the need to use an antimicrobial agent that complements the control of bacterial plaque seems clear continuously and effectively. Plaque formation is a dynamic and orderly process. On a clean tooth surface, the primary plaque formers, streptococci, establish first, the presence of which is essential for the adhesion of other bacterial species. The following species provide the means and the creation of a suitable environment for the adhesion and proliferation of other microorganisms, increasing the plaque in bacterial quantity and quality. In the ordered formation of plaque, processes of bacterial adherence, proliferation and division are involved. Mechanical

cleaning acts on the tooth surface, not sterilizing the surface, but limiting the bacterial mass, leaving a small non-pathogenic plaque that is compatible with gingival health.[6]

The microorganisms of the oral cavity commonly organize themselves into biofilms in the form of dental plaque adhered to the hard and soft tissues of the mouth. Bacterial biofilm is considered an extremely important factor in the etiology of dental and periodontal diseases, as well as in post-surgical infections.[7]

Chlorhexidine gluconate is accepted as the "gold standard" among chemotherapeutic agents used in mouthwash. 0.2% chlorhexidine gluconate with high antibacterial properties. It has been reported that the daily use of the preparation can reduce *S.mutans* colonization by 30-50%[1]. However, in addition to the plaque control feature of the agent, the search for chemical or herbal preparations that can show similar efficacy continues[2]. Chlorhexidine is the most effective antiseptic, but it should be used in short periods of 2 weeks in situations where hygiene is diminished, however studies show that its long-term use does not produce bacterial resistance, although the appearance should be controlled of staining with periodic prophylaxis. The composition of chlorhexidine has an influence, the rest of the components being important since, like sodium fluoride, the effectiveness of chlorhexidine decreases[3]. Listerine has been shown to aid in daily plaque control, so it can be a valid mouthwash for maintenance patients. Hexetidine does not prove to have relevant results in plaque control, although this improves when zinc salts are added. The nonalcoholic formulation of chlorhexidine is just as effective as the alcoholic solution. Components added to chlorhexidine to decrease the staining index can decrease the effectiveness of it.The aim of this in vitro study is to examine the antibacterial effects of six different mouthwashes on *Streptococcus mutans, Enterococcus faecalis, Bacillus subtilis, Lactobacillus casei,* and*Staphylococus aureus.*

MATERIALS AND METHODS

The contents of the materials we use to examine the antibacterial effects of different types of mouthwash on *Streptococcus mutans, Enterococcus faecalis, Bacillus subtilis, Lactobacillus casei, Staphylococus aureus* are shown in Table.1

Table 1: Heavy mouthwashes whose antibacterial activities were evaluated, their ingredients and manufacturers along with their pH

Mouth wash	Ingredients	pH of each of the mouthwash solutions tested.	Manufacturing Company
1	Active Ingredient: Hydrogen Peroxide 1.4%. Purpose: Oral debriding agent/oral wound cleanser Inactive Ingredients: Water, Sorbitol, Propylene Glycol, Poloxamer 338, Polysorbate 20, Flavor, Sodium Saccharin, FD&C Blue 1	6.2	Colgate-Palmolive Co, Jordan
2	Aqua, alcohol, sorbitol, poloxamer 407, benzoik asit, Sodyum saccharin, eucalyptol, methyl salicylate, aroma, tymol, menthol, sodium benzoate, CI 47005, CI 42053	5.6	DR. TICHENOR'S, USA
3	Sodium fluoride 0.02% (0.01% w/v fluoride ion) (Anticavity). Inactive Ingredients:Water, alcohol (8%), Aqua, sorbitol, peg-40 hydrogenated castor oil, trisodium phosphate, PVMMA copolymer, sodium lauryl sulfate, aroma, benzyl alcohol, phenoxy ethanol, sodium saccharin, sodium floride, lecithin, glycerin, limonene CL74160	6.9	Listerine, Johnsons & Johnsons Jordan
4	chlorhexidine gluconate, Eucalyptol 0.092% (antiplaque/antigingivitis)Menthol 0.042% (antiplaque/antigingivitis)Methyl salicylate 0.060% (antiplaque/antigingivitis)Thymol 0.064% (antiplaque/antigingivitis). Aqua, glycerin, aroma, cetyl pyridinium chloride, poloxamer407, methyl paraben, sodium saccharin, cinnamal, propylparaben, eugenol, CL 42090	3.5	Listerine, Johnsons & Johnsons Jordan
5	chlorhexidine gluconate, Cetylpyridinium chloride 0.07% Inactive Ingredients: Water, Glycerin, Flavor, Poloxamer 407, Sodium Saccharin, Methylparaben, Sucralose, Propylparaben, Blue 1	3.7	Crest, Jordan
6	GARGAROL® is composed of a unique combination of Chlorhexidine Gluconate 0.2% and other ingredients. It is an Antiseptic, antibacterial, anti-inflammatory, and analgesic mouthwash. GARGAROL® is ideal for prophylaxis and treatment for mouth infections, periodontitis, denture stomatitis, gingivitis and minor aphthous ulcers.	6.1	GARGAROL, US Group, Jordan

In the study using agar diffusion method, antibacterial effect was investigated with standard bacterial strains delivered to England National Collection of Type Cultures, Central Public Health Laboratory and Amman Hygiene Institute Culture Collection, Jordan. The bacteria used are as in Table.2.

Table 2: Standard lyophilized bacterial strains

Microorganisms (by the order of use) ™	
Steptecoccus mutans	ATCC 35668
Enterococcus faecalis	BAA-2128
Bacillus subtilis	ATCC 23857
Lactobacillus casei	ATCC 393
Staphylococcus aureus	ATCC 23235

The lyophilized bacterial strains were carefully opened in the Microbiology Laboratory of Jordan University, Faculty of Dentistry under sterile conditions. The suspensions of the strains specified in Table.2 were prepared according to Mc-Farland 0.5 standard and were cultivated in broth and kept in an oven at 37°C for 24 hours. Then, with the help of sterile swabs, each sowing was applied to the 7% sheep blood Müller-Hinton agar media by repeating it twice. 7 nests of 5 mm diameter and 2 mm depth, one of which was left empty for control, were opened on the petri dishes prepared with standard sterile perforators. Six different mouthwashes were used in the study conducted in ten different petri dishes. Gargles were placed in these holes with macropipettes untouched by human hands and kept in an oven at 37°C for 24 hours. At the end of 24 hours, the inhibition zone thicknesses around the samples inside the petri dishes were evaluated by measuring millimetrically with a caliper.

Statistical analysis

Kruskal Wallis and Mann Whitney U Test were used to compare the antibacterial effects of six different mouthwashes on five identified microorganism species.

RESULTS

The antibacterial effects of the tested mouthwash on *Streptococcus mutans, Enterococcus faecalis, Bacillus subtilis, Lactobacillus casei, Staphylococus aureus* are shown in Table.3. According to the data obtained, all of the mouthwashes used showed a different degree of antibacterial activity on microorganism species compared to the control group ($P < 0.05$). When the inhibition zone thicknesses formed by the mouthwashes at the end of 24 hours were compared, it was seen that the preparation number 6 showed a statistically significant high antibacterial activity on *S mutans, E faecalis, L.casei, and S.aureus*. ($P < 0.05$).

Table 3: Statistical evaluations of the inhibition zone thicknesses of 6 different mouthwashes tested on 5 different micro-organisms using the Kruskal Wallis and Mann Whitney U Test.

	Mean diameter of inhibition zones ± standard deviation						
	1. Mouthwash-A	2. mouthwash-B	3. mouthwash-C	4. mouthwash-D	5. mouthwash-E	6. mouthwash-F	Control -G
S.mutans	2.0 ± 0.70	3.7 ± 0.40	2.6 ± 0.89	2.2 ± 0.47	2.8 ± 0.44	6.6 ± 1.4	0
	BCFG	ADFG	AFG	BFG	FG	ABCDEG	ABCDEF
E.faecalis	1.7 ± 0.27	0.12 ± 0.04	0.54 ± 0.27	0.1 ± 0.11	0.14 ± 0.05	4.6 ± 0.54	0
	BCDEFG	AFG	AFG	AFG	AFG	ABCDEG	ABCDEF
B.subtilus	0.9 ± 0.96	0.12 ± 0.04	1.6 ± 0.84	2.1 ± 0.51	1.7 ± 0.44	3.2 ± 0.44	0
	FG	DEFG	FG	BG	BFG	ABCEG	ABCDEF
L. casei	2.8 ± 0.83	0.28 ± 0.04	0.84 ± 0.35	1.2 ± 0.68	1.12 ± 0.71	5 ± 0.70	0
	BCF	AGF	AFG	FG	FG	ABCDEG	ABCDEF
S.aureus	2.4 ± 0.54	0.38 ± 0.37	1.9 ± 0.41	2.0 ± 0.01	2.1 ± 0.20	4.2 ± 0.44	0
	BFG	ACDEFG	BFG	BFG	BFG	ABCDEG	ABCDEF

* Capital letters used in the table are used to explain the statistical differences between groups on the horizontal plane.
** ($P < 0.05$) (n: 10).

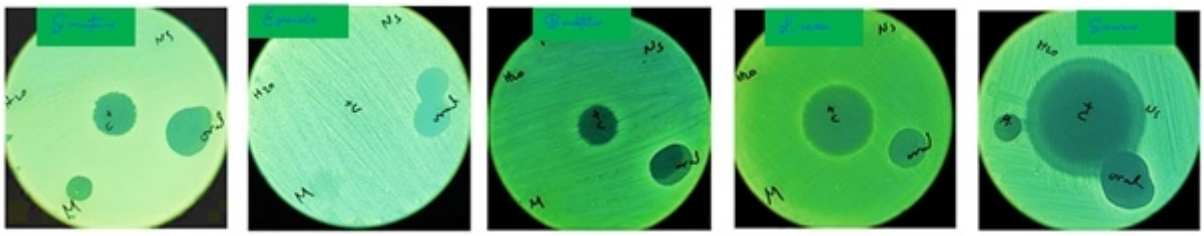

Figure 1: Antimicrobial activity of five microorganisms in selected mouthwash solutions

Figure 1 shows that selected mouth cleansers produced antimicrobial activity against*S.mutans, E.faecalis, B.subtilus, L. casei and S.aureus* Microorganisms

DISCUSSION

Oral cavity surfaces are constantly colonized by microorganisms. There are more than 250 species in a millilitre of saliva, and it has been shown that the dominant species are streptococci, which also play an important role in caries formation[8]. Although mechanical cleaning is the way to prevent the colonization of microorganism species in the oral cavity; Patient compliance, faulty applications, presence of complicated prosthesis and / or appliances in the mouth are the limitations of effective cleaning. Therefore, the use of chemotherapeutic agents for the purposes of supporting oral hygiene and providing antimicrobial efficacy is among the oral care recommendations. It has been shown that the different gargages used in this study have different levels of anti-microbial activity on the specified microorganism species.S.mutans is one of the precursor microorganism species involved in biofilm structure and plays an important role in dental caries formation. Paying attention to approaches to preventing caries formation is still a popular suggestion in the world, and pharmacological or chemotherapeutic methods are used for this purpose[9]. In this study, it was observed that especially the preparation numbered 2 and 6 had a high effect on *S. mutans* and this difference was significant (P <0.05). Two-numbered preparation; It was introduced to the market as an OTC in the USA in 1914 and was approved by ADA in 1988. Patients are recommended to gargle a 20 ml solution twice a day for 30 seconds, and it is in category 1 (safe and effective) by the FDA[10]. The active agents in its content are thymol, menthol, eucalyptol, methyl salicylate and essential oils. Thanks to methyl salicylates, the preparation also has an anti-inflammatory effect[11]. Preparation number six contains sodium fluoride as an active ingredient. WHO's recommendation for fluoride mouthwashes for the prevention of caries; there are two types of daily use: 0.05% sodium fluoride mouthwash (230 ppmF) or 0.2% sodium fluoride mouthwash (900ppmF) once a week or every 15 days. Systematic reviews and meta-analysis suggest that fluoride mouthwashes can be used to prevent caries.While 26% reported that it was effective, its relationship with the initial decay amount, the amount of fluoride exposure and the frequency of gargling was not investigated[12]. Although this in-vitro study showed that the preparation was antibacterial, the fact that the study was conducted under in vitro conditions and the factors that could change the mouthwash efficacy such as mouth environment, temperature, pH, saliva flow could not be imitated. It is one of its limitations.

Although it is known that chlorhexidine gluconate, which is accepted as the gold standard in chemical plaque elimination, is a broad spectrum anti-microbial, it is not indicated for long-term use, it causes staining on teeth and restorations, has side effects such as taste disturbance, supragingival calculus formation. It limits its use. The most important feature that distinguishes chlorhexid from other antiseptics is that it has a long-term release profile (high substantivity) by adhering to tissues when applied in the oral cavity. Thanks to this feature, it shows a more effective antiseptic effect compared to other antiseptic agents[13]. In our study, preparations numbered 3 and 5 contain the active ingredient of chlorhexidine gluconate. Although it is effective in terms of antibacterial activity, as shown in table 3, the inhibition zone remained at a low level compared to some of the preparations with which it was compared. Although this result is surprising, it can be considered that the feature that makes chlorhexidine superior to gluconate is its high substantivity. In this context, it can be considered that it is clinically important to investigate the antibacterial properties of the aforesaid preparations as well as the duration of their effectiveness in the real network environment.

Although the support of chemotherapeutic agents is important in terms of oral hygiene, long-term use of chemical ingredients is controversial. Therefore, recent studies have focused mostly on natural and herbal ingredients. One of the herbal ingredients with known anti-plaque activity is the Sanguarina active ingredient. This substance is an alkaloid produced from the plant Sanguinarinacanadensis. The formula of the preparation is 0.01% sanguinarine and 0.2% zinc chloride. Gargle form contains 11.5% alcohol at pH 4.5. Paste pH is 5.2, but both products are not ADA approved. When chlorhexine gluconate is compared with the active ingredient preparations in terms of its side effects, some patients do not have side effects such as staining, calculus formation or disturbance in the sense of taste[14].

In a study, it was reported that when using 0.03% sanguinaria solution, the plaque decreased by 40% and gingivitis by 25% compared to the placebo group. In a study conducted by Moran et al., 0.2% CHX and Viadent were compared and CHX was found to be more effective in reducing plaque and gingivitis[13]. In this study, no herbal preparations were used due to the lack of a commercial form in our country's market.

CONCLUSION

Although the preparations used in our study were shown to have antibacterial properties in *in-vitro* conditions, oral conditions play an important role in ensuring clinical efficacy. In this sense, research with experimental protocols to provide an environment that can mimic oral conditions may be more useful. In addition, the efficacy of mouthwashes, which can be easily removed from the mouth environment with saliva flow and daily routines (eating and drinking, etc.) should also be considered.

Anti-plaque mouthwashes can be used as a support for mechanical plaque removal in order to maintain oral hygiene effectively. Therefore, preparations with minimized side effects or herbal products can be placed in the patient's oral care routine. The clinician should carefully analyze the patient's oral hygiene status and provide appropriate oral care recommendations.

Future clinical studies to evaluate the effectiveness of mouthwashes will assist the clinician in determining the benefit that can be provided, and the oral hygiene levels of patients can be increased with the use of appropriate preparations.

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