

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFECTIVENESS OF PROBIOTIC MOUTHWASH WITH CHLORHEXIDINE AND POVIDONE IODINE MOUTHWASH – AN IN VITRO STUDY

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Abstract

INTRODUCTION: Live microbes that, when given in sufficient doses, have a positive effect on health are known as probiotics (WHO). Probiotics affect dental health in a number of ways, including by influencing the immune system, preventing infections from adhering to the oral mucosa, and promoting competitive exclusion. Probiotics were also used against chromogenic bacteria which are the predominant cause for extrinsic tooth stains. **AIM OF THE STUDY:** The purpose of this research was to examine the antibacterial activity of probiotic mouthwashes in individuals suffering from periodontitis. [In the fight against periodontal disease]. **MATERIALS & METHODS:** Five participants were enrolled in this research by collecting saliva samples. They took notes on clinical variables like probing pocket depth, clinical attachment level, gingival index, plaque index, and more. Three mouthwashes were tested for periodontal bacteria using three different antimicrobial media. Microbial investigations were done. **RESULTS:** The mouthwashes differed in their effects on the expanded inhibition zone. Statistical analysis reveals that the chosen mouthwashes are distinct from one another. **CONCLUSION:** In this research, the antibacterial effect of Probiotic mouthwash was shown to be greater compared to the other two mouthwashes tested.

Keywords: Antimicrobial Mouthwash, Chlorhexidine, Dental Plaque, Microbial Sensitivity Tests, Oral Bacteria.

INTRODUCTION

Dental plaque is considered as one of the initiating factor for periodontal diseases. There are various methods to reduce the amount of dental plaque including mechanical and chemical methods. Chemical methods include the use of mouthwashes and they have a significant role in reducing the amount of microbial plaque. An antimicrobial solution, also known as mouthwash or mouth rinse, is a useful home care method for better dental hygiene. Cavities, gingivitis, and foul breath are all caused by bacterial plaque, but anti-plaque mouth rinses eliminate these germs. Fluoride is an anti-cavity agent that is used in several mouthwashes. Chlorhexidine (CHX) digluconate is considered the gold standard (1). It is still the gold standard when it comes to antiplaque mouthwashes. When compared to Gram-negative bacteria, it works better against Gram-positive ones. Reason being, it comes with a host of unwanted consequences, such as a shift in flavor, more tartar buildup, discoloration, cavities, and problems with dentures and other oral equipment (2). Clinical studies show oral bacteria developing resistance to CHX when used for long duration. Also, CHX has many side effects on oral cavity³ and so usage of chlorhexidine mouthwash is limited. Researchers Challacombe et al. showed that nasal spray and povidone iodine (PVP-I) mouthwash might help prevent dentists and dental assistants from contracting the COVID-19 virus. At 15, 30, and 60 seconds, virucidal activity against

SARS-CoV-2 in clean and dirty conditions was tested in a BSL-3 laboratory at the Tropical Infectious Diseases Research and Education Center (TIDREC), University of Malaya, Malaysia. The two concentrations of BETADINE Gargle and Mouth Wash were tested, one undiluted and the other at a 1:2 dilution. According to the World Health Organization (2014), probiotics are living microbial cultures that, when given to the host in sufficient doses, improve the host's health. Probiotic bacteria are beneficial microbes that help keep the microbiome in check. Probiotics affect dental health in a number of ways, including by influencing the immune system, preventing infections from adhering to the oral mucosa, and promoting competitive exclusion. Caries, candidiasis, xerostomia, burning mouth syndrome, periodontal diseases, and many other oral disorders may be alleviated with the use of probiotics (3). The most common kind of extrinsic teeth stains are caused by chromogenic bacteria, however probiotics are now being used as a mouthwash to treat gingivitis and periodontitis (4). This research aims to assess the antimicrobial impact of several mouthwashes on individuals with chronic generalized periodontitis. Specifically, it will examine the effects of chlorhexidine, Povidone iodine, and probiotics.

MATERIALS AND METHODOLOGY

After patients with chronic generalized periodontitis gave their informed permission, plaque samples were taken for this in vitro investigation. Inclusion in the research was contingent upon the participants' being 40 or older, free of any preexisting conditions, and having a clinical examination with a probing depth more than 5 mm. People who had been using corticosteroids, antibiotics, or anti-inflammatory medications for the last six months were not included. This research also did not include any subjects who used mouth rinse. The test microorganism selected for this study is *Streptococcus mutans* and blood-agar culture was used to grow the streptococcus strains.



Fig 1: Clinical image of generalized chronic periodontitis

Study design:

A comparative study to evaluate the efficacy of probiotic mouthwash, povidone iodine and chlorhexidine mouthwash for treatment of chronic generalized periodontitis.

Sample size:

A total of six teeth were chosen at random, air-dried, and isolated using cotton rollers after patients who met the inclusion criteria were found using systematic random sites. The buccal and lingual surfaces of the teeth were used to obtain supragingival plaque samples, which were then placed in a sterile tube with 1ml of saline. Shortly after that, the sample is sent out for microbiological analysis.



Materials Used:

In the present study two mouth washes has been used – Chlorhexidine (**Fig 2**): commercially available non-alcoholic 0.2% CHX mouthwash (Hexedine® 160 ml ICPA Health Products Ltd, Mumbai, India.), Probiotic mouthwash (Lemon mint antiplaque rinse **Fig 3**). Antimicrobial effectiveness of various mouthwashes was assessed by using Disc Diffusion Assay:



Fig 4

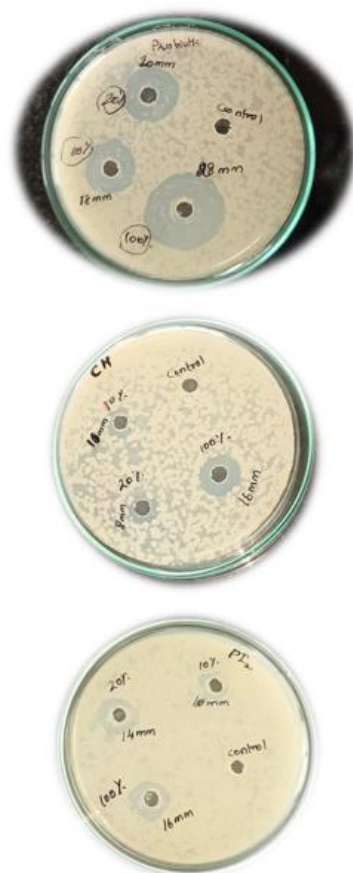


Preparation

The bacterial strains were incubated at 37°C for 24 hours after being subcultured on sterile Muller Hinton Broth. After a 5-minute centrifugation at 10,000 rpm, the resulting broth was rinsed with sterile saline. The turbidity was then corrected to 0.5 McFarland standard, which is 1×10^8 CFU/ml at 600 nm.

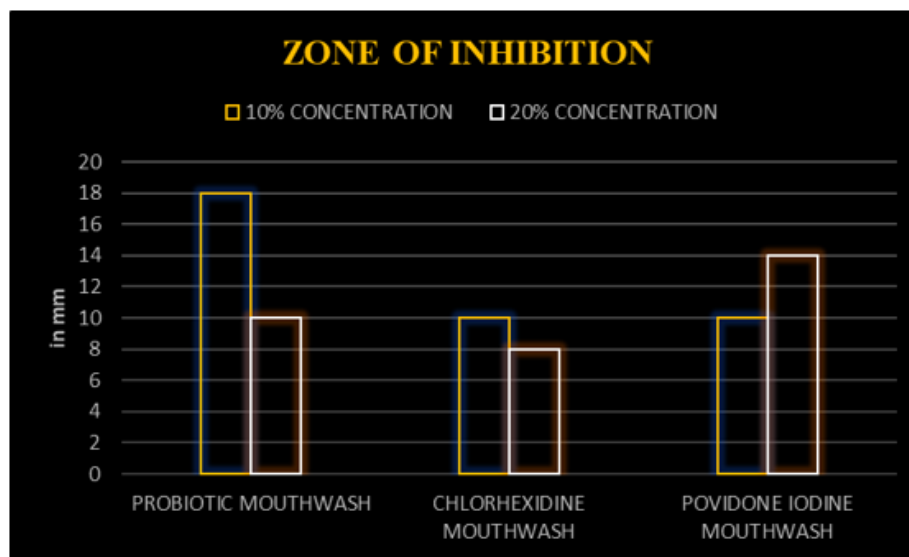
Disc Diffusion Assay: (Fig 5, Fig 6, Fig 7)

Bacterial inoculum was cultured on MULLER HINTON AGAR plates and 6 mm diameter well were made and impregnated with 10µl, 20µl, 30µl, 40µl, 50µl of sample and streptomycin (10µl) used as control. We evaluated the inhibitory zone width after 24 hours of incubation at 37°C, and we used CLSI standards to figure out what that meant. A one-way analysis of variance test was used for statistical analysis of the inhibition zone diameters around different solution concentrations.



RESULTS

Chlorhexidine and probiotic mouthwash inhibited the growth of *S. mutans* but probiotic showed better results than chlorhexidine. The zone of growth inhibition for *S. mutans* was maximum for **probiotic strain 10% (18 mm) and 20%(20mm) and minimum for chlorhexidine 10% (10 mm), 20% (8mm) & povidone iodine 10% (10mm), 20% (14mm)** . All findings were found to be statistically significant when comparing the groups. The bar chart (8a, 8b, 8c) shows the clearing zone. When pitted against chlorhexidine, probiotic proved to be much more efficient against *S. mutans*.



DISCUSSION

Bacteria that inhabit the mouth are the principal culprits behind the majority of dental problems (5). The bacterium responsible for the most common dental disease, dental caries, namely *S. mutans*, was the focus of this investigation. The goal of both preventative and curative dental care is to keep the mouth free of harmful microbes. By preventing the proliferation of oral harmful microbes, natural compounds with antimicrobial characteristics bolster these endeavors. Beneficial effects on host health may be achieved by the administration of probiotic bacteria, which are live microorganisms. According to Hull et al (6) *Lactobacillus* and *Bifidobacterium* are the earliest genera of probiotic bacteria. The presence of probiotics in the mouth microflora improves the efficacy of probiotic therapy and shows that they are compatible with the oral environment. As far as dental hygiene products go, probiotic treatment is the most promising option (7). Lozenges, mouthwash, gelatin, powder, straws, and pills are some of the many delivery vehicles for probiotics. Nowadays, it's advised to use a probiotic mouthwash that contains live bacteria. There is no evidence of toxicity, they are safe for use in the mouth, and they are not resistant to antibiotics. While several preliminary research have shown that probiotics have beneficial benefits, confirmation would need a large number of clinical trials. This research found that compared to conventional chlorhexidine mouthwashes, probiotic mouthwash significantly reduced microbial growth and had a wider growth inhibition zone. Oral prophylaxis with CHX or hydrogen peroxide was compared in a research by Horster and Korf for patients with mandibular fractures. When compared to hydrogen peroxide, the findings demonstrated that 0.2% CHX was superior at preventing plaque development (8). While the probiotic mouthwash shown stronger antibacterial effects, CHX was more effective in suppressing microorganisms in this investigation. In the metaverse, VR and AR can create immersive simulations that demonstrate the proper use of chlorhexidine in patient care, enhancing understanding and adherence to best practices for infection control (9).

Periodontitis patients were found to have microorganisms in 40% of cases before to scaling and root planing (SRP) and 23% after the procedure, according to research by Mombelli et al. (1994). It has been found that oral microorganisms may persist after

scaling and root planning (10). One of the most common periopathogens is bacteria, and this fact alone demonstrates that bacteria are resistant to SRP therapies. Compared to other mouthwashes, probiotic mouthwash was able to substantially suppress its proliferation in the current investigation. In addition to SRP therapy, a probiotic mouthwash may help decrease oral bacteria.

Researchers Twetman et al. looked at the effects of probiotic gum on gingival inflammation and inflammatory mediator levels in gingival crevicular fluid (GCF) in individuals with gingivitis. They discovered that probiotics reduced inflammation and lowered levels of IL-8, tumor necrosis factor-alpha, and IL-1B in GCF (11). In a delicate equilibrium, probiotics may stick to surfaces and replace harmful bacteria with good ones. Research by Jothika M. et al. examined the effects of fluoride, chlorhexidine, and probiotic mouthwashes on the levels of *Streptococcus mutans* in plaque over a short period of time and found that all three were effective in reducing *S mutans* levels. In terms of efficacy, probiotic mouthwash is on par with other popular brands like chlorhexidine and sodium fluoride (12). Some of the ways in which probiotics exert their effects include regulating the immune response, degrading pollutants, competing with other microorganisms for nutrients or binding sites, and producing antimicrobial compounds (13). To inform future clinical trials, this study's findings may be useful. To back up claims of probiotic effectiveness, in vivo research are required, since in vitro studies have certain limitations.

CONCLUSION

The many negative effects of CHX make it unsuitable for long-term use, yet it may be given as an adjuvant for shorter durations. In this age of evidence-based medicine, it is standard practice to do extensive testing on any product with potential clinical use to establish its biocompatibility with the oral cavity's tissues and its efficacy in promoting and sustaining high standards of oral hygiene and disease prevention. This research found that probiotics were more effective than chlorhexidine in preventing *S mutans* infections. To find out how effective it is, however, a bigger sample size is needed.

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