



# **Antimicrobial efficiency of mouth rinses against the different pathogen of periodontal diseases**

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## **Abstract**

Oral health complications include dental caries, endodontic infections, and periodontal diseases which contribute greatly to the impairment of oral health and a big financial burden to people all over the world. In this investigation, there was the use of 4 mouthwashes under the names Colgate Plax, Listerine Cool Mint, Hexidine and Clohex Antiplaque washer. The antibacterial action of each mouthwash was determined using the well diffusion technique against three dental pathogens which included *Streptococcus mutans*, *Porphyromonas gingivalis* and *Candida albicans*. All the mouthwashes showed significant activities in all pathogens, but Hexidine showed the best tolerance of maximum zone of inhibition in *S. mutans* (23 mm) and *Candida albicans* (24 mm) with the Clohex Antiplaque and Colgate Plax product showing an intermediate zone of inhibitions in all the tooth infections. The lowest zone of inhibition of *S. mutans* (22 mm) and *Candida albicans* (23 mm) was observed in Listerine Cool Mint. All the mouthwashes showed a great influence on all the infections. In all pathogens, Hexidine has exhibited a higher MIC and MBC value. The mouthwashes in general showed similar antibacterial activity towards all of the pathogens that were tested. Although all the experienced mouthrinses are composed of chlorhexidine and most of them include cetylpyridinium chloride, it's the results indicate significant differences in their antibacterial activity. Hexidine and Clohex Antiplaque showed the noteworthy against to all oral microbes.

**Keywords:** Oral health, Periodontal diseases, Antimicrobial activity, Mouthrinses, Minimum inhibitory concentration

## Introduction

Oral health is critically influenced by the presence and activity of various microbial pathogens. Poor oral hygiene leads to the accumulation of microbes in the mouth, resulting in harmful effects. Typically, oral microbes are mostly commensally that help preserve oral wellbeing and protect against bad bacteria (Yumoto et al., 2019). Dental problems generally fall into three main categories: dental plaques, dental caries, and periodontal diseases (Manupati and Wolter, 2011). Dental caries, or tooth decay, is a bacterial disease affecting the enamel, dentin, and cementum. The decay is caused by acids produced by bacteria, leading to the breakdown of teeth (Silk, 2014). Tooth decay, can also result in awful breathing and an unpleasant taste. In advanced cases, contamination may multiply from the teeth to nearby area of mouths, potentially leading to a degrade the tooth, which can be either partial or complete. The disease is primarily caused by acidogenic bacterial species such as *Streptococcus mutans*, *Lactobacillus*, *Porphyromonas gingivalis*, and *Actinomyces*. These bacteria utilize the sugars into acidic products within colorless film of bacteria on the tooth outside, leading to the dissolution of calcium phosphate in the enamel and, accordingly, the development of tooth decay (Baelum et al., 1997).

Periodontal disease is an infectious process of supporting structures of the tooth, which begins with the initial and reversible stage of gingivitis (an erythematous, edematous, and hemorrhagic gingival tissue) and then could proceed to periodontitis, where it could cause serious damage to the periodontal tissues and the osseous structures, thus, causing tooth loss. (Jabbarifar et al., 2015). This is the most prevalent form which is gingivitis, inflammation of the gums. Microorganisms that are linked to such conditions are *Streptococcus mutans*, *Escherichia coli*, *Staphylococcus aureus* and *Candida* species (Gamboa et al., 2004). Dental plaque is easy to remove and easily soft yet when not eliminated it becomes hard and extremely hard to clean and thus causes problems to oral health.

An oral rinse or a mouthwash is a non-sterile aqueous mouth solution that is used to keep the mouth clean. It is mainly used because of its deodorant, invigorating, antiseptic, and antibacterial effect. Mouthwashes can be used to decrease the number of microbes in the oral cavity, to remove food debris, and to regulate plaque formation as well as to eliminate bad breath, leaving mouth and taste good and fresh. Mouthwashes may be cosmetic (primarily they are used to mask bad smell and provide freshness) or therapeutic (they may include active ingredients such as chlorhexidine, essential oils, or fluoride to prevent or treat oral diseases). Considering that the use of proper mouthwash, along with brushing and flossing, are useful to maintain the overall oral health, a regular use of a proper mouthwash plays a role in that. Active ingredients have been studied in finding their usefulness in decreasing dental plaque as well as streptococci that are bacteria related to the dental caries. Mouthwashes which are antimicrobials acting and they assist in maintaining oral hygiene. Mouthwash, also known as oral rinse, is a liquid used to rinse the teeth, gums, and mouth. It typically contains antiseptics that target harmful bacteria residing between teeth and on the tongue. The antimicrobial effect of mouthwash comes from the active antimicrobial agents formulated within it. Although many dentifrices claim antimicrobial properties, limited research has been conducted to validate these claims (Nogueira-Filho et al., 2002).

Chlorhexidine digluconate is regarded as the fabulous disinfectant of the mouthrinses. The 0.2% solution of chlorhexidine is the typical dose that is applied to prevent the formation of biofilm in the mouth. CHX attach to the bacteria surface because it is ionic which damages their cell membranes and leads to the death of the microbes. Rinses that contain fluoride e.g. use sodium fluoride (NaF) at 0.2% concentration are rinsed once per week and 0.05% daily. Popularity of these rinses is attributed to their cheapness, convenience, and their taste. Mouth rinses with cetylpyridinium chloride (CPC) have supplementary advantages when administered as an adjection to oral health procedures that assist

in decreasing the amount of plaque and gingivitis inflammation. Active ingredients such as fluoride, chlorhexidine or CPC are found in therapeutic rinses to aid in the prevention of dental disease, as well as purely aesthetic benefits. Based on the limited available literature, The current research was meticulously crafted to examine the in vitro antimicrobial effectiveness of four distinct mouthrinses targeting four periodontal microbial pathogens, namely *Streptococcus mutans* (*S. mutans*), *Porphyromonas gingivalis* (*P. gingivalis*) and *Candida albicans* (*C. albicans*).

## Materials and Methods

To conduct this study, there were four commercial products of mouthwash which were bought in one drugstore in Adayar Chennai.

### Antimicrobial activity

Four commercially available mouthwashes—Colgate Plax, Listerine Cool Mint, Hexidine Mouth Wash, and Clohex Antiplaque Mouthwash against three oral pathogens: *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Candida albicans*. Microbial culture was done by the use of proper culture media to help in the growth of these organisms. Kirby-Bauer Disk Diffusion test which is a widely acceptable technique to find out antibiotic resistance, or susceptibility, was used to do the antibacterial activity. In this test, the discs that have been impregnated with the mouthwash are put on the cultured bacterial plates and the areas of inhibition that surround the discs are measured in order to measure antimicrobial efficacy.

### The Kirby-Bauer Disk Diffusion Test:

The cultures of the test microbial were adjusted to  $1.5 \times 10^8$  CFU/ml, 0.5 McFarland standard preparation by using sterile distilled water. The antimicrobial sensitivity of the test culture to the four mouthwashes was determined using the Kirby-Bauer disc diffusion method. For this, 20 ml of sterilized Mueller-Hinton agar, cooled to 45°C, was poured into sterile petri plates and allowed to solidify. A bacterial lawn was prepared

by evenly spreading 100 µl of the inoculum ( $1.5 \times 10^8$  CFU/ml) over the entire agar surface using a sterilized spreader. Using a sterile 8mm cork-borer, equidistant wells were made in each agar plate, into which the mouthwashes were introduced to observe zones of inhibition.

In the experiment, five wells were prepared on each agar plate. Using a micropipette, 100 µL, 75 µL, 50 µL, 25 µL and control of the full concentration of each of the four mouthwashes were added into the wells on the petridishes inoculated with the microbial strains. The plates were incubated at 37°C for 24 hours. After incubation, antimicrobial activity was assessed by measuring the diameter of the zones of inhibition (in millimeters) around each well. The size of the inhibition zone indicated the antibacterial or antifungal potential of the mouthwash against the specific microorganism. The experiment was performed in triplicate to ensure accuracy and reproducibility. A measuring scale was used to measure to the nearest whole millimeter the diameter of the zone of inhibition. Upon the measurements, the petridishes were coded to reveal what mouthwash was tested, and the measurements were compared.

### Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

All bacterial strains were sourced from the American Type Culture Collection (ATCC). For the MIC determination, 15 test tubes were prepared, each containing one mL of sterile tryptic soy broth. Serial dilutions of each mouthwash were then prepared; 1 mL of these dilutions was added to the respective tubes. A bacterial suspension standardized to  $1.5 \times 10^8$  CFU/mL, equivalent to the 0.5 McFarland standard, was prepared from the standard strains. The tubes with the diluents of the mouthwash were inoculated using this suspension to determine the concentration at which growth of the microbes was prevented. The tubes were incubated and kept at a temperature of 37°C in 48 hours. Incubation was followed by testing the tubes on the basis of turbidity, which is used as a

measure of the growth of microbes. MIC was considered as the final dilution of mouthwash at which no turbidity was detected, which meant that it inhibited growth of microbes. In order to establish the Minimum Bactericidal Concentration (MBC) the clear (non-turbid) tubes were sub-cultured on solid media and allowed to incubate further, in order to measure the growth of the microbe. The minimal concentration of mouthwash that allowed the avoidance of growth of the microbes on the solid media was considered to be the MBC. The same procedure was carried out on the rest of bacterial strains that had been tested and the MIC and MBC values of each mouthwash strained to compare the efficacy of the antimicrobial (Forbes, 2007).

## Results

The mouthwashes were assessed for zones of inhibition, with Hexidine showing high zones of inhibition, indicating excellent antimicrobial activity. Chlohex and Colgate Plax also showed zones of inhibition, suggesting equally good activity against all tested microorganisms. Chlorhexidine and Listerine exhibited an average ability to inhibit microbial growth. Figure 1 showed inhibit the growth of disease-causing microorganisms activity of the four mouthrinses against *Streptococcus mutans* in Mueller-Hinton agar by measuring the zones of inhibition. The sterile distilled water control did not produce any zones of inhibition against the tested microorganisms. Reduced bacterial efficacy of the mouthwashes was depicted significantly as in Figure 1. The Kirby-Bauer disc diffusion procedure was used and the maximum zone of inhibition was 23 mm against *S. mutans* and *C. albicans* at 100 µl concentration, as shown in Table 1. Even though some of the mouthwashes had changes in their inhibition zones after 24 hours although the changes were occasionally minor, most of the antimicrobial activity was recorded in the first 24 hours of incubation with all four mouthwashes being tested.

Figure 1 results of the well diffusion test indicate that all the mouthwashes had an impact that inhibited the growth of the microorganisms tested

in a concentration-dependent manner. All the four tested mouthwashes had the greatest number of inhibition zones with Hexidine followed by Clohex Antiplaque ( $22 \pm 0.77$  mm) and Colgate Plax ( $20 \pm 0.90$  mm) (Table 1). These data show that Hexidine and Clohex Antiplaque were the most effective in the growth inhibition of all the examined microorganisms. The effectiveness of such mouthwashes is explained by the chlorhexidine, which is a popular oral disease antibacterial agent. The chlorhexidine had marginally bigger inhibition zones although the differences were not very significant meaning that other mouthwashes could be an effective alternative or adjunct in controlling oral infections. Hexidine demonstrated the highest antimicrobial activity overall, with a maximum inhibition zone against *C. albicans* (24 mm), followed by *S. mutans* (23 mm), and the smallest inhibition zone against *P. gingivalis* (20 mm) at different concentrations, with the strongest effect observed at full strength (Fig. 1). The inhibitory activity of Colgate Plax against *S. mutans* increased at full strength when measured after 24 hours of incubation. For the other mouthwashes, the zone diameters remained almost the same after 24 hours. However, all mouthwashes showed a slight increase in the zone of inhibition when the concentration was increased concentration.

All the tested mouthwashes demonstrated bactericidal effects and were effective in stopping the growth of the microorganisms. The Minimum Inhibitory Concentrations (MICs) of Colgate Plax, Listerine Cool Mint, Hexidine, and Clohex Antiplaque mouthwashes for *S. mutans* were 0.13, 0.15, 0.12, and 0.13 µg/mL, respectively (Table 2). The Minimum Bactericidal Concentrations (MBCs) for the same mouthwashes against *S. mutans* were 0.22, 0.27, 0.20, and 0.21 µg/mL, respectively. The MICs and MBCs for other bacterial cultures are also detailed in Table 1, with the lowest MIC values observed for Hexidine and Clohex Antiplaque across all bacterial strains tested. These two mouthwashes showed the greatest antimicrobial effect. Among the bacterial strains, *P. gingivalis* exhibited the highest resistance to chlorhexidine mouthwashes, while

*C. albicans* was the most sensitive to these treatments.

The four distinct mouthwashes were arranged in order of antimicrobial efficacy using statistical ranking procedures after the two distinct methods for evaluating the mouthwashes'

antimicrobial potential were finished. Their efficacy against the three tested microorganisms varies greatly, according to the results. Hexidine mouthwash was found to be the most effective antimicrobial mouthwash among the three tested microorganisms. Clohex and Colgate Plax also demonstrated excellent levels of activity.

Table -1 Concentration of Mouthwash against *Microorganisms*

<i>Microorganisms</i>	Mouthwash	Concentration			
		25µl	50µl	75µl	100µl
<i>Streptococcus mutans,</i>	Colgate Plax	8±0.32	12±0.77	15±0.63	20±0.90
<i>Porphyromonas gingivalis,</i>		7±0.37	11±0.41	14±0.07	18±0.91
<i>Candida albicans</i>		9±0.27	12±0.36	17±0.42	23±0.48
<i>Streptococcus mutans,</i>	Listerine Cool Mint	9±0.28	11±0.32	15±0.42	17±0.64
<i>Porphyromonas gingivalis,</i>		7±0.28	10±0.37	14±0.046	15±0.60
<i>Candida albicans</i>		11±0.45	12±0.77	15±0.30	18±0.36
<i>Streptococcus mutans,</i>	Hexidine	10±0.32	11±0.51	19±0.52	23±0.77
<i>Porphyromonas gingivalis,</i>		9±0.37	12±0.30	17±0.06	20±0.79
<i>Candida albicans</i>		11±0.27	12±0.30	20±0.36	24±0.42
<i>Streptococcus mutans,</i>	Clohex Antiplaque	10±0.32	13±0.51	19±0.52	22±0.77
<i>Porphyromonas gingivalis,</i>		8±0.32	11±0.30	17±0.06	21±0.7
<i>Candida albicans</i>		12±0.27	10±0.30	20±0.36	23±0.42

Table -2 MIC and MBC of Mouthwash against *Microorganisms*

<i>Microorganisms</i>	Mouthwashes µg/ml							
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	Colgate Plax		Listerine Cool Mint		Hexidine		Clohex Antiplaque	
<i>Streptococcus mutans,</i>	0.13	0.22	0.15	0.27	0.12	0.2	0.13	0.21
<i>Porphyromonas gingivalis,</i>	0.14	0.23	0.16	0.29	0.13	0.23	0.14	0.22
<i>Candida albicans</i>	0.11	0.2	0.13	0.25	0.1	0.21	0.12	0.21

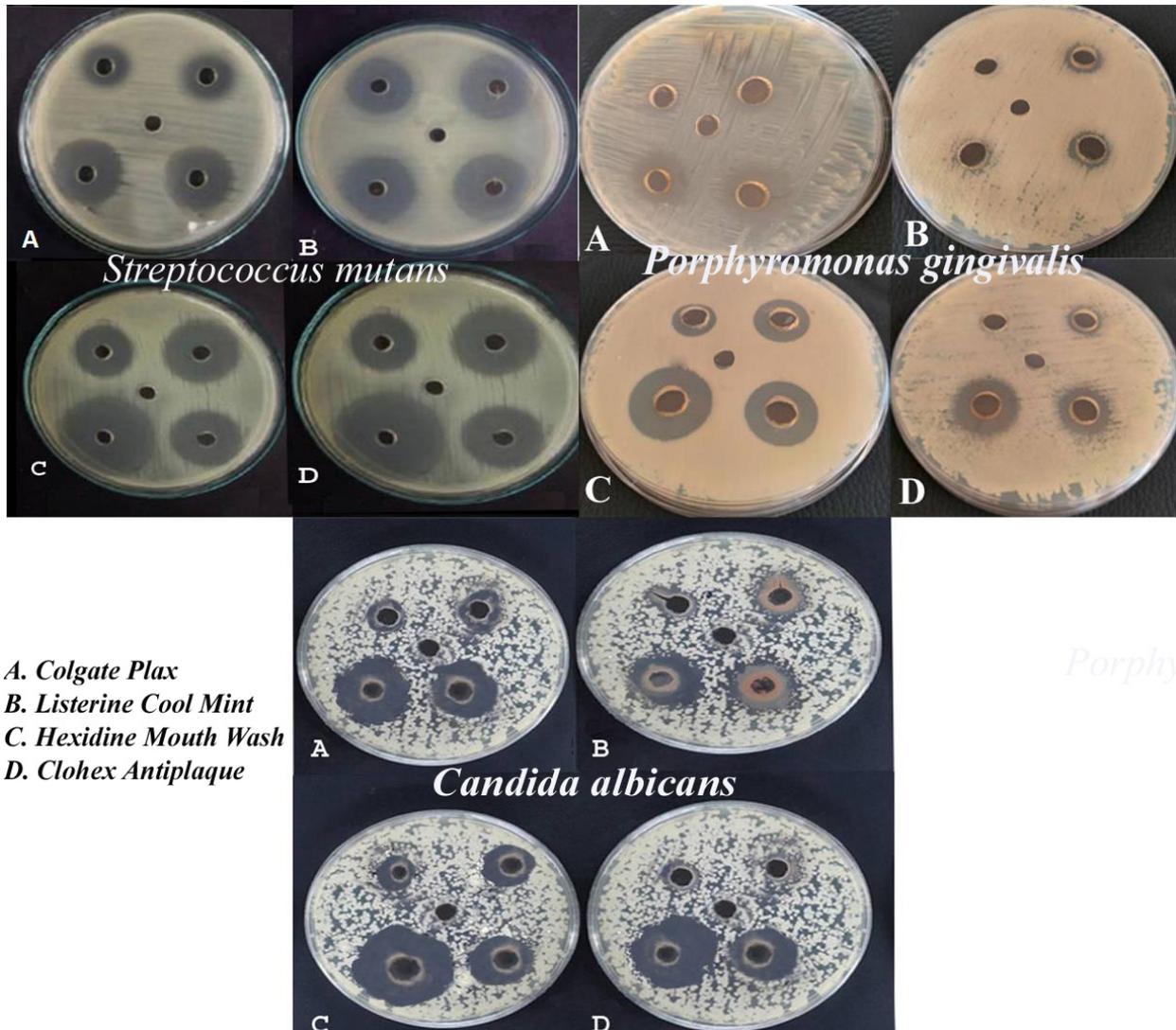


Figure 1 : Antimicrobial effect of Mouthwashes against different dental pathogens

## Discussion

Chlorhexidine mouthwash is the most popular of the different types of mouthwash. An immediate and most likely transient bactericidal effect appears to be the mechanism of action of a chlorhexidine mouthwash, which is followed by a prolonged bacteriostatic action that depends on the antiseptic absorbed by the pellicle coating the tooth surface. Children use mouthwash containing sodium fluoride the most frequently. According to Sundar and Rao (2011), this mouthwash is known to be a strong anticariogenic agent and to effectively reduce dental cavities.

Additionally, the effectiveness of sodium fluoride mouthwash was not superior to that of chlorhexidine mouthwash in the current study (Malhotra et al., 2011).

Dental caries may be avoided by using an antimicrobial agent in the oral cavity, which would stop microorganisms from growing. Both fluoride and chlorhexidine are frequently used anticariogenic mouthwashes. Compared to the most widely used pharmaceutical mouthwash, the mouthwash preparations are thought to have a moderate level of efficacy and to be less harmful. The primary offender among cariogenic microorganisms is thought to be *S. mutans*.

According to a synopsis of *S. mutans* involvement in the pathophysiology of dental caries, the bacteria first attach themselves to the surface of the enamel through adhesion-mediated attachment, where they ferment sucrose and form hydrophobic bonds.

It is well known that the bacteria's strong adherence and aggregation to the tooth surface is further encouraged by this sucrose metabolism, which leads to the production of acid and the development of cavities (Jeon et al., 2011). It is well known that an individual's oral microbial population is in balance.

Losing this equilibrium can allow opportunistic microbes to multiply and start disease processes. Thus, mouthwashes with the big microbial inhibition zone and thus, the strong antibacterial and antifungal properties—may not always be better than those with inhibition zones of smaller diameter. (Barry and Thornsberry, 1991).

According to Charles et al., chlorhexidine is regarded as a safe and efficient antiseptic for reducing plaque levels of streptococci and gingivitis (2004). The antimicrobial activity of 0–12% chlorhexidine has been thoroughly studied, and the results of this investigation are in line with previous research. (Zanela et al., 2002), since Periogard prevented the growth of bacteria with MIDs ranging from 6 to 25 percent to 1 to 56 percent. The combination of essential oils (eucalyptol, menthol, thymol, and methyl salicylate) in Listerine mouthrinse has been shown to be effective in reducing gingivitis and dental plaque. In contrast to the results of earlier research, Listerine did not have an inhibitory effect on any of the strains tested in this study (Fine et al., 2000). Therefore, it is clear from the overall results that different mouthwash with chlorhexidine gluconate as the active ingredient had distinct antimicrobial properties.

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