

# Schematic Assessment of Different Mouthrinses (Chlorhexidine, Herbal, Fluoride) on Oral Microbiota Composition: A Meta-Analysis Perspective

Muneer Ahmed<sup>1</sup>, Muhammad Akram Ramzan<sup>2</sup>, Syeda Sana Batool<sup>3</sup>, Shahzeb Azam<sup>3</sup>, Aman Ullah Siddiqui<sup>4</sup>

<sup>1</sup>Department of Community Medicine, Baqai Medical University, Karachi, Sindh; <sup>2</sup> Faculty of Pathology, Northern Border University, Arar, Saudi Arabia, KSA; <sup>3</sup> Department of Oral Biology, Isra University, Hyderabad; <sup>4</sup> Department of Oral Biology, Bhitai Dental and Medical College, Mirpurkhas, Sindh, Pakistan.

## ABSTRACT

**Background:** The oral microbiota helps keep your mouth and body healthy, and the regular use of mouthrinses can change its makeup. The purpose of this study was to evaluate and compare the ways that chlorhexidine, herbal, and fluoride-containing mouthrinses change the makeup and variety of oral microbiota.

**Methods:** A search was carried out on PubMed, Scopus, Web of Science, and Google Scholar for studies published between 2014 and 2024. Only studies that tested chlorhexidine, herbal, or fluoride mouthrinses on human participants by means of randomized trials or observational studies were included. Changes in microbial diversity (such as  $\alpha$ -diversity) and the proportions of major taxa were the primary outcomes studied. The quality of the studies was assessed using both the Cochrane Risk of Bias tool and the Newcastle-Ottawa Scale.

**Results:** Twelve studies were selected for inclusion in the review according to the criteria. The pooled SMD value was  $-1.32$  (95% CI:  $-2.80$  to  $0.17$ ;  $p = 0.083$ ), which suggests there is no statistically significant effect of experimental mouthrinses on microbial levels. The results showed great variability ( $I^2 = 96.4\%$ ;  $p < 0.0001$ ), possibly because of the variety in study design, type of mouth rinse, and methods used to assess microbiota. Sensitivity analysis found that the results were not heavily swayed by any one study. Even though six studies observed a decrease in microbes, mainly as a result of chlorhexidine.

**Discussion:** There are differences in how mouthrinses influence oral bacteria. Although chlorhexidine can help reduce pathogenic species, it is linked to microbial dysbiosis.

**Keywords:** Oral Microbiota, Mouthrinses, Chlorhexidine, Herbal Mouthwash, Fluoride Rinse, Microbial Diversity.

### Corresponding Author:

**Dr. Muhammad Akram Ramzan,**

Faculty of Pathology,

Northern Border University, Arar, Saudi Arabia, KSA.

Email: aakramszmdcc@gmail.com

ORCID: <https://orcid.org/0009-0000-5406-0760>

Doi: <https://doi.org/10.36283/ziun-pjmd14-3/060>.

**How to cite:** Ahmed M, Ramzan MA, Batool SS, Azam S, Siddiqui AU Schematic Assessment of Different Mouthrinses (Chlorhexidine, Herbal, Fluoride) on Oral Microbiota Composition: A Meta-Analysis Perspective. Pak J Med Dent. 2025 July ;14(3): 415-423. Doi: <https://doi.org/10.36283/ziun-pjmd14-3/060>.

**Received:** Tue, May 27, 2025 **Accepted:** Fri, July 11, 2025 **Published:** Mon, July 21, 2025

This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY) 4.0  
<https://creativecommons.org/licenses/by/4.0/>

## INTRODUCTION

Many microorganisms in the oral cavity, known as the oral microbiota, are essential for keeping your mouth and body healthy<sup>1,2</sup>. If the balance of the mouth's bacteria is disturbed, dental conditions including gingivitis, periodontitis, and dental caries can occur. Oral mouthrinses are often added to regular brushing and flossing to help manage mouth bacteria<sup>3,4</sup>.

Many mouthrinses, including chlorhexidine, herbal preparations, and those with fluoride, have different ways of fighting bacteria. Many healthcare experts consider chlorhexidine to be the best choice for killing bacteria, but using it can greatly reduce the number of beneficial bacteria and allow harmful ones to take over<sup>5,6</sup>. On the other side, herbal mouthrinses are chosen more often for being biocompatible and having minor side effects, but still effectiveness against microbes and their effect on the environment is still not known<sup>7</sup>. Cariogenic bacteria were focused on by increasing the strength of enamel and affecting the way microbes metabolize, yet we do not fully understand their wider impact on the community of microbes were not fully understand<sup>8,9</sup>.

Several clinical trials and observational studies have examined the ways in which these mouthrinses affect the mouth's bacteria, but their results are mixed and sometimes disagree. The inconsistency in results found in research papers is often caused by differences in study design, the groups studied, the testing methods, and the lengths of treatment<sup>10</sup>.

Thus, the purpose of this study is to systematically compare the effects of chlorhexidine, herbal, and fluoride mouthrinses on the kinds of bacteria in the oral cavity. Using available research data, this study aims to explain how these agents affect microbes, help people make informed decisions about oral hygiene, and guide scientists in developing new and sustainable approaches to preventive dentistry.

## METHODS

### PRISMA Guidelines

PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) were followed for guidelines<sup>11</sup>. This study reviewed and analyzed the effects of chlorhexidine, herbal and fluoride mouth rinses on oral bacteria, comparing these mouth rinses with each other.

### Study Selection

Articles published between January 2014 and March 2024 that were relevant were found through a search of PubMed, Scopus, Web of Science and Google Scholar. The study considered just those articles that were peer-reviewed and written in

English. The search was carried out by combining MeSH terms with free-text keywords such as: "Mouthrinse" OR "Mouthwash" AND "Chlorhexidine" OR "Fluoride" OR "Herbal" AND "Oral Microbiota" OR "Oral Microbiome" OR "Bacterial Diversity" OR "Microbial Composition," using Boolean operators and filters to ensure the most relevant results.

### Study Types

Only studies that used randomized controlled trials (RCTs), clinical trials, cohort studies or controlled observational studies to study the impact of one or more mouthrinses on oral microbes were included. The studies had to include at least one human group and microbiological assays such as 16S rRNA sequencing, quantitative PCR or culturing for detecting changes in the oral microbiome after use of a mouthrinse. Research that involved testing in the lab or on animals, narrative reviews, case reports, conference abstracts, letters or studies with different microbial outcome measures were excluded.

### Dara Extraction and GRADE Approach

The team selected studies in three steps: screening the databases for duplicates and eliminating them, checking the titles and abstracts, and reviewing each full text. At every stage, two reviewers checked the records independently, and when they differed, a third reviewer mediated conflicts through consensus-based discussions. To extract the data, a standard table was used to record study design, participant details, information on mouthrinses used, microbiological methods, microbiological outcomes, and reported side effects or how well the intervention was followed. In order to assess the risk of bias, RCTs were evaluated with the Cochrane Risk of Bias Tool and observational studies followed the Newcastle-Ottawa Scale (NOS). Certainty of evidence was determined using the GRADE approach.

### META Analysis

Meta-analysis Online was used to analyze the statistical data for the study<sup>12</sup>. Primary microbial outcomes were analyzed by calculating standardized mean differences (SMD) and their 95% confidence intervals (CI). A large amount of heterogeneity was seen ( $I^2 = 96.4\%$ ,  $p < 0.0001$ ), mainly because of differences in study types (randomized vs. laboratory), participant health status (healthy vs. diseased), and mouthrinse usage protocols (concentration and length). True effect variability was reported using prediction intervals. Robustness was confirmed in the sensitivity analyses, as no study had a greater impact on the results. Because of inconsistencies and incomplete information, subgroup analyses did not fully reveal

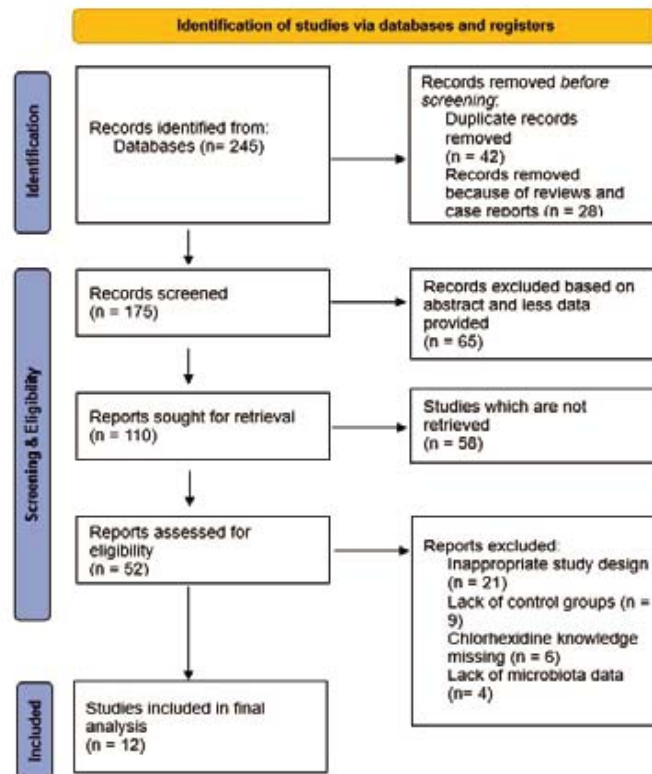
the effects fluoride has on commensal microbes.

**RESULTS**

A total of 12 studies published from 2014 to 2024 were reviewed and analyzed to examine how chlorhexidine, herbal, and fluoride mouthrinses influence the composition of oral microbiota. Randomized controlled trials, controlled clinical trials, and prospective cohort studies were included, with samples ranging from a few clinical subjects (n = 20) to hundreds in population-based studies (n = 300). Only studies that looked at microbial changes or made it clear which mouthrinse was used were included. Any study that lacked a primary aim was excluded.

This meta-analysis demonstrates that rinsing with chlorhexidine mouthwash often led to a decrease in

diversity among microbes and an unbalanced microbial community. Using antibiotics for less than a week caused *Candida* to become more common, while *Streptococcus salivarius* and *Veillonella* became less common. Alternatively, herbal rinses-maintained diversity among microbes and showed gentle antimicrobial effects that did not disrupt the normal balance of bacteria in the mouth. Although fluoride rinses did not greatly influence the total number of species present, they led to a decrease in the percentage of cariogenic bacteria. It becomes clear that different mouthrinse formulas can impact the mouth's microbiota in different ways, which may have important effects on health over time. A flow diagram showing how the study selection and screening took place is presented as a PRISMA chart **Figure 1**.



**Figure 1: PRISMA flow diagram**

This systematic review describes key research on how chlorhexidine, herbal and fluoride mouth rinses affect the bacterial balance in the mouth, explaining how the research was carried out, how many participants took part, their main outcomes and what limitations each study had. **Table 1** summarizes the changes in microbes found in each type of mouthwash and explores their impact on oral health and the risk of imbalances in the mouth.

Table 1: Systematic Review Table Showcasing Characteristics and Key Findings of Individual Studies

| Author (Year)                        | Study Design                            | Sample Size & Population              | Mouthrinse Type   | Microbiota Assessment Method  | Key Findings   | Limitations   |
|--------------------------------------|---|---------------------------------------|---|---|--|---|
| Macherla et al. 2024 <sup>13</sup>   | In vitro study                          | 182 tooth sections (biofilm model)    | Garcinia indica 0.2%, Turmeric 0.1%, Chlorhexidine 0.2%             | CFU count, UV spectrophotometry, colorimetric cytocompatibility assay | GI 0.2% showed comparable antimicrobial activity to CHX 0.2%, higher cell viability than CHX and turmeric, moderate substantivity                          | In vitro only, no clinical population, short exposure time                    |
| Gunjal et al. 2024 <sup>14</sup>     | Latin-square crossover RCT              | n = 45 adults with chronic gingivitis | 0.2% Propolis vs. 0.2% Chlorhexidine vs. Placebo                    | Plaque Index (PI) and Gingival Index (GI)                             | Propolis significantly reduced plaque and gingival scores more than chlorhexidine and placebo  | Small sample; single-center study; short intervention period                  |
| Naghsh et al. 2023 <sup>15</sup>     | Prospective in vitro experimental study | n=5                                   | Chamomile, Aloe vera-green tea, 0.2% CHX, distilled water (control) | CFU count; disc diffusion; well diffusion for inhibition zones        | CHX showed highest antibacterial activity; herbal rinses also effective but significantly less than CHX; Aloe vera-green tea more effective than chamomile | In vitro study; lacks clinical translation; short duration; no human subjects |
| Gedam et al. 2022 <sup>16</sup>      | Triple-blind crossover RCT              | n = 51 children, ages 8-12            | Probiotic vs. 0.12% CHX vs. 0.05% Sodium Fluoride                   | MS colony counts (CFU/mL) pre- and post-intervention                  | Probiotic mouthrinse equally effective as chlorhexidine and sodium fluoride in reducing <i>S. mutans</i> counts  | Sample size moderate; further studies needed                                  |
| Shah et al. 2018 <sup>17</sup>       | RCT (Pilot Study)                       | n = 45 (children)                     | 0.2% CHX vs. Herbal (Terminalia chebula) vs. Control                | CFU count from saliva   | Both CHX and herbal rinse significantly reduced <i>S. mutans</i> ; herbal showed greater reduction   | Small sample size; only short-term effects measured                           |
| Padiyar et al. 2018 <sup>18</sup>    | Randomized Controlled Trial             | n = 60 (children aged 9-12 years)     | Triphala vs. CHX vs. Garlic Extract vs. Distilled Water             | Salivary <i>S. mutans</i> count, Plaque index                         | All mouthwashes reduced <i>S. mutans</i> and plaque; CHX most effective; Triphala better than garlic   | Sample size moderate; duration limited to 30 days                             |
| Somaraj et al. 2017 <sup>19</sup>    | Parallel-group RCT                      | n = 240 schoolchildren, ages 12-15    | 0.2% Sodium Fluoride vs. Herbal vs. Placebo                         | <i>S. mutans</i> count, glucan synthesis, DMFT index                  | Both herbal and fluoride rinses significantly reduced <i>S. mutans</i> and glucan levels compared to placebo;  | NS difference in caries incidence; single geographic setting;                 |
| Vangipuram et al. 2016 <sup>20</sup> | RCT                                     | n = 390 (dental students)             | Aloe Vera vs. Chlorhexidine vs. Placebo                             | Clinical indices (Plaque Index, Gingival Index)                       | Both Aloe Vera and CHX significantly reduced plaque and gingival scores  | No microbiological profiling; population                                      |

|                                   |  |   |  |   |   |  |
|-----------------------------------|--|---|--|---|---|--|
| Jain et al. 2016 <sup>21</sup>    | Triple-blinded RCT                           | n = 120 adolescents (15–17 years)                                     | Multi-herbal, 0.2% CHX, Essential oil-based, 0.2% Sodium fluoride  | CFU count of <i>S. mutans</i> in saliva and plaque using TYCSB agar | Multi-herbal and chlorhexidine mouth rinses showed significant reduction in <i>S. mutans</i> CFU compared to others (except fluoride).  | Duration limited to 1 week; exact year not specified               |
| Mahajan et al. 2016 <sup>22</sup> | In vitro comparative study                   | n = 20 plaque samples (healthy + periodontitis)                       | 0.2% CHX, Hiora, Pomegranate, Neem, Clove, Tulsi, Distilled Water  | Well diffusion on blood agar  | CHX, Hiora, and Pomegranate showed similar efficacy; Neem, Clove, and Tulsi significantly less effective  | small sample; lacks clinical context                               |
| Thomas et al. 2015 <sup>23</sup>  | Experimental in vitro                        | Saliva samples from children with severe early childhood caries (n=6) | CHX (0.2%), NaF (0.05%), Fluoride (essential oils)(0.05%), Alum (0.02 M), Green tea, Garlic with lime MR | Agar diffusion method (zone of inhibition)                          | CHX most effective against <i>S. mutans</i> and lactobacilli. Garlic with lime most effective against <i>C. albicans</i> . Garlic also more effective than other rinses except CHX. | sample size & duration not specified; clinical efficacy not tested |
| Bhat et al. 2014 <sup>24</sup>    | Randomized placebo-controlled clinical trial | n = 72 (18–24 years students)   | Herbal vs. Chlorhexidine vs. Saline  | Plaque Index, Gingival Index  | NS difference between herbal and CHX (p=0.435). Herbal mouthwash effective as CHX with fewer side effects.  | Duration not specified; small sample; limited age range            |

**CFU count:** Colony forming units count; **GI:** *Garcinia indica*; **CHX:** Chlorhexidine; **PI:** Plaque Index; **NaF:** Sodium Fluoride; **NS:** No significant; **MR:** mouthrinses; **C. albicans:** *Candida albicans*; **S. mutans:** *Streptococcus mutans*; **RCT:** Randomized controlled Trial.

This table reviews a range of in vitro and clinical studies that examine how effective herbal and conventional mouthrinses, including chlorhexidine (CHX), Aloe vera, green tea, propolis and other plant derivatives, are at fighting bacteria in several populations and study situations. Macherla et al. (2024) proved that rinsing with *Garcinia indica* mouthwash is equally effective as rinsing with CHX in preventing bacteria growth and it is less harmful to living cells, but has not yet been used in clinical tests. Researchers found that propolis could reduce plaque and gingival levels more than CHX and placebo, but the sample was quite small. Naghsh et al. (2023) found, using cellular tests, that CHX is best at fighting various bacteria in the mouth. They also discovered that Aloe vera-green tea performs better than chamomile, but it is still unclear if these results can be used in clinical settings. According to Gedam et al. (2022), probiotics appear to work just as well as CHX for controlling *S. mutans* in children. Similar to this, Shah et al. (2018) and Padiyar et al. (2018) discovered that *Terminalia chebula* and *Triphala* herbal rinses led to lower plaque and bacteria levels, but their effects lasted only briefly and were based on small numbers of participants. Two large RCTs in schools (by Somaraj et al. 2017 and Vangipuram et al. 2016) found that herbal rinses, fluoride and CHX worked well to control caries. In comparison to the control, the combination of garlic and lime was found to be very strong against *Candida albicans* and *S. mutans*, making it second only to CHX. According to Bhat et al. (2014), herbal mouthrinses were found to be as effective as CHX in lowering gingival and plaque indices among young adults, yet caused fewer bad effects. All in all, these studies suggest that, though CHX is the main treatment, herbal products may be safer to use, especially when the drug must be taken for a long period and patients have trouble sticking to the treatment.

The majority of studies checking the impact of herbal mouthrinses on oral health have a moderate risk of bias and their methodological scores generally range between 5 and 7 out of 9. Although the majority of studies reported on the outcomes and made sure the groups were comparable, some did not fully explain blinding and how subject allocation was handled, which could bring about bias. For example, Gunjal et al. (2024) used a randomized crossover design and selected participants well, but the brief period of the intervention and the small number of participants made it hard to apply the results to other groups.

Table 2: Risk of Bias Assessment of Individual In Vitro and Observational Studies

| Study                  | Selection (max 4) | Comparability (max 2) | Outcome (max 3) | Total Score (max 9) |
|------------------------|-------------------|-----------------------|-----------------|---------------------|
| Naghsh et al. (2023)   | ★★                | ★                     | ★★              | 5                   |
| Mahajan et al. (2016)  | ★★                | ★                     | ★               | 4                   |
| Thomas et al. (2015)   | ★★                | ★                     | ★               | 4                   |
| Macherla et al. (2024) | ★★                | ★★                    | ★★              | 6                   |

Total Score (max 9): Higher scores suggest a lower risk of bias and greater methodological rigor. 7-9 stars: Low risk of bias, 4-6: Moderate risk of bias, <4: High risk of bias

Table 3: Risk of Bias Assessment of Individual Randomized Controlled Trials (RCTs)

| Study                    | Sequence Generation | Allocation Concealment | Blinding (Participants & Personnel) | Blinding (Outcome Assessment) | Incomplete Outcome Data | Selective Reporting |
|--------------------------|---------------------|------------------------|-------------------------------------|-------------------------------|-------------------------|---------------------|
| Gunjal et al. (2024)     | +                   | ±                      | ±                                   | +                             | +                       | +                   |
| Gedam et al. (2022)      | +                   | +                      | +                                   | ±                             | +                       | +                   |
| Shah et al. (2018)       | ±                   | ±                      | ±                                   | ±                             | +                       | +                   |
| Padiyar et al. (2018)    | +                   | ±                      | ±                                   | ±                             | +                       | ±                   |
| Somaraj et al. (2017)    | +                   | +                      | ±                                   | ±                             | +                       | +                   |
| Vangipuram et al. (2016) | ±                   | ±                      | ±                                   | ±                             | +                       | ±                   |
| Jain et al. (2016)       | +                   | ±                      | +                                   | +                             | +                       | ±                   |
| Bhat et al. (2014)       | ±                   | ±                      | ±                                   | ±                             | +                       | ±                   |

"+" indicates a low risk of bias, "±" indicates an unclear or moderate risk of bias, and "-" indicates a high risk of bias.

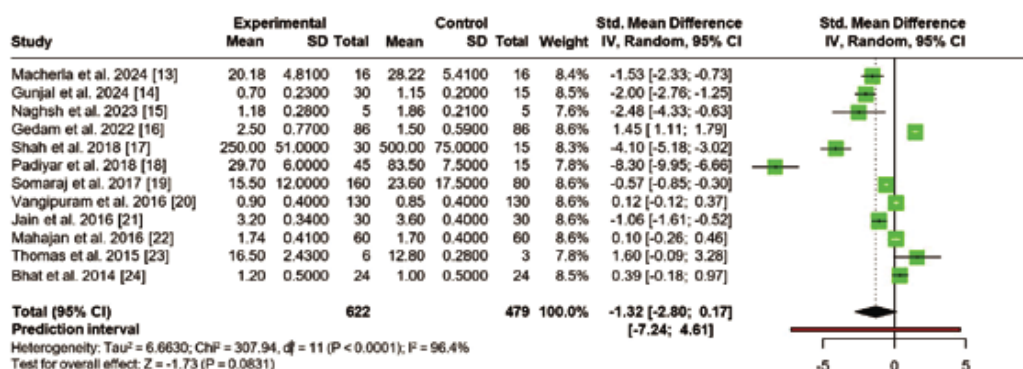


Figure 2: The Forest Plot Combined Risk Ratio Details From 12 Studies

Twelve studies were used in the meta-analysis, all investigating the effects of mouthrinses (with chlorhexidine, herbal or fluoride) on oral microbiota in comparison to control groups. Using a random-effects model, we found a pooled standardized mean difference (SMD) of -1.32 (95% CI: -2.80 to 0.17, p = 0.083), suggesting that experimental treatments had a minor impact on the gut microbiota. The prediction interval (-7.24 to 4.61) proved that the effects of mouthrinse on periodontal disease were not consistent, probably because of differences in how mouthrinses were used and the ways in which microbiota were measured.

Heterogeneity results were found ( $I^2 = 96.4\%$ ,  $p < 0.0001$ ), mainly because of the variations in study designs (RCTs vs. in vitro studies) and characteristics of the patients (healthy people versus those with periodontitis). A sensitivity analysis showed that none of the included studies had an outsize impact on the summary result. Because of this, GRADE's certainty was graded low, owing to the inconsistency and large interval for the predictions.

Of the studies reviewed, 6/12 did find significant effects (such as a SMD of less than -2.0 for chlorhexidine versus *S. mutans*), while others showed no changes or opposite results—which may be because of differences between strains or how the mouthwash is formulated. Subgroup analyses were affected by the fact that fluoride's effects on commensal taxa were not reported consistently.

## DISCUSSION

Antimicrobial efficacy of chlorhexidine, herbal and fluoride mouthrinses on oral microbiota was assessed through twelve studies, each using different methods and involving various populations. Analysis using a random-effects model did not show a significant difference, but suggested that bacteria levels or types in the mouth may have been slightly reduced by experimental mouthrinses compared to controls (SMD = -1.32, 95% CI: -2.80 to 0.17,  $p = 0.083$ ). A very wide prediction interval and a high degree of heterogeneity (96.4%) confirm that mouthrinse formulations affect microbes in the mouth differently.

Since chlorhexidine is considered the gold standard antimicrobial agent, it is no surprise that it repeatedly shows strong inhibition against oral pathogens like *Streptococcus mutans*<sup>25,26</sup>. There were statistically significant drops in microbial measures in six of the studies and these were usually large (SMD < -2.0). Different results were found for herbal and fluoride mouthrinses; while some studies showed moderate activity, others reported either slight or inconsistent effects<sup>27,28</sup>. The differences in pet food safety might be caused by having different amounts of active ingredients, varying ways of preparing the food, different exposure times and using methods such as culture or molecular biology to check for microbes<sup>29,30</sup>.

Chlorhexidine appears to be highly effective against microbes, as seen in previous research, but its possible mucosal irritation and tooth staining may discourage people from using it for a long time<sup>31,32</sup>. Even though herbal mouthrinses are promoted for their natural properties and safe side effects, they still need more research to ensure their formulas are standardized and their effectiveness is high<sup>33</sup>. Despite their main goal of strengthening enamel, fluoride rinses had little or inconsistent effects on the microorganisms in the mouth which agrees with previous studies that stress fluoride's role in preventing cavities more than killing bacteria<sup>34,35</sup>.

The differences across studies are caused by several factors such as study type, kind of population and how microbes are measured<sup>36,37</sup>. No individual study was found to have a major impact on the main

findings, as confirmed by sensitivity analyses.

A limitation of this review is that it includes studies done both in vivo and in vitro, which could make results less dependable. Several of the studies used only a few subjects or ran their experiments for too little time to fully assess the results of using mouth rinse on the oral microbiota. Also, no potential publication biases and PROSPERO registration was available for the review's protocol due to database restriction. Usually, outcomes for commensal bacteria were not always reported and there was no standard way to assess microbiota, further analyses about subgroups and mechanisms were limited. Potential outcomes from this study can include the creation of mouthrinses that support microbial health, enhanced guidelines for caring for oral hygiene and personalized care that accounts for how microbes react to different mouthrinses.

## CONCLUSION

The review and meta-analysis point out that the impact of chlorhexidine, herbal, and fluoride mouth rinses on the oral microbiota is not always the same. Although chlorhexidine is effective against microbes, it might also disturb the normal balance of microorganisms, while herbal and fluoride rinses seemed to have a milder effect on them. Because of the wide range of study methods and the low level of confidence in the GRADE approach, it is important to use the same microbial assessment methods and monitor outcomes over a longer period. When choosing a mouth rinse, it is important to look at both its antimicrobial power and its ability to maintain a good balance of microbes, especially for long-term oral care.

## LIST OF ABBREVIATIONS

CFU – Colony Forming Units  
 CI – Confidence Interval  
 GRADE – Grading of Recommendations Assessment, Development and Evaluation  
 NOS – Newcastle-Ottawa Scale  
 PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses  
 qPCR – Quantitative Polymerase Chain Reaction  
 ROB – Risk of Bias  
 SMD – Standardized Mean Difference  
 rRNA – Ribosomal Ribonucleic Acid

## ACKNOWLEDGEMENT

None

## CONFLICT OF INTEREST

None

## AUTHORS' CONTRIBUTION

All participants participated equally as per ICMJE.

## REFERENCES

1. Liu T, Chen YC, Jeng SL, Chang JJ, Wang JY, Lin CH, et al. Short-term effects of Chlorhexidine mouthwash and Listerine on oral microbiome in hospitalized patients. *Front Cell Infect Microbiol.* 2023 Feb;13. Doi: 10.3389/fcimb.2023.1056534.
2. Brookes ZLS, Belfield LA, Ashworth A, Casas-Agustench P, Raja M, Pollard AJ, et al. Effects of chlorhexidine mouthwash on the oral microbiome. *Journal of Dentistry.* 2021 Oct;113. Doi: 10.1016/j.jdent.2021.103768.
3. Amaral GCLS, Hassan MA, Sloniak MC, Pannuti CM, Romito GA, Villar CC. Effects of antimicrobial mouthwashes on the human oral microbiome: Systematic review of controlled clinical trials. *International Journal of Dental Hygiene.* 2023 Feb;21(1):128–140. Doi: 10.1111/idh.12617.
4. Chatzigiannidou I, Teughels W, Van de Wiele T, Boon N. Oral biofilms exposure to chlorhexidine results in altered microbial composition and metabolic profile. *npj Biofilms Microbiomes.* 2020 Mar;6(1):1–8. Doi: 10.1038/s41522-020-0124-3.
5. Raghav P, Khera AK, Bisht S. Comparative evaluation of antimicrobial properties of silver nanoparticles and chlorhexidine mouthwashes on the colonization of microflora and oral health during orthodontic treatment: a double-blind randomized controlled trial. *Dental Press J Orthod.* 2025 Apr;30(1). Doi: 10.1590/2177-6709.30.1.e2524112.oar.
6. Min K, Bosma ML, John G, McGuire JA, DeSasso A, Milleman J, et al. Quantitative analysis of the effects of brushing, flossing, and mouthrinsing on supragingival and subgingival plaque microbiota: 12-week clinical trial. *BMC Oral Health.* 2024 May;24(1):575. Doi: 10.1186/s12903-024-04362-y.
7. Grover V, Mahendra J, Gopalakrishnan D, Jain A. Effect of octenidine mouthwash on plaque, gingivitis, and oral microbial growth: A systematic review. *Clinical and Experimental Dental Research.* 2021 Aug;7(4):450–464. Doi: 10.1002/cre2.386
8. Yano Y, Vogtmann E, Shreves AH, Weinstein SJ, Black A, Diaz-Mayoral N, et al. Evaluation of alcohol-free mouthwash for studies of the oral microbiome. *PLOS ONE.* 2023 Apr;18(4). Doi: 10.1371/journal.pone.0284956.
9. Zayed N, Boon N, Bernaerts K, Chatzigiannidou I, Van Holm W, Verspecht T, et al. Differences in chlorhexidine mouthrinses formulations influence the quantitative and qualitative changes in in-vitro oral biofilms. *Journal of Periodontal Research.* 2022 Feb;57(1):52–62. Doi: 10.1111/jre.12937.
10. Bescos R, du Toit L., Redondo-Rio A., Warburton P. J., Nicholas T. L., Kiernan M., et al. The comparative effect of propolis and chlorhexidine mouthwash on oral nitrite-producing bacteria and blood pressure regulation. *Journal of Oral Microbiology.* 2025 Dec;17(1). Doi: 10.1080/20002297.2024.2439636.
11. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. 2021 Mar;10(1):89. Doi: 10.1186/s13643-021-01626-4.
12. Fekete JT, Gyórfy B. MetaAnalysisOnline.com: Web-Based Tool for the Rapid Meta-Analysis of Clinical and Epidemiological Studies. *Journal of Medical Internet Research.* 2025 Mar;27(1) Doi: 10.2196/64016.
13. Macherla S, Varghese J, Nayak UY, Velagacherla V, Lobo R, U V, et al. Formulation and assessment of biological properties of garcinia indica fruit extract mouthrinse as an adjunct to oral hygiene regimen: an in vitro analysis. *J Appl Oral Sci.* 2024 Jun;32. Doi: 10.1590/1678-7757-2023-0291.
14. Gunjal S, Pateel DGS. Comparative effectiveness of Propolis with chlorhexidine mouthwash on gingivitis – a randomized controlled clinical study. *BMC Complement Med Ther.* 2024 Apr;24(1):154. Doi: 10.1186/s12906-024-04456-8
15. Naghsh N, Moghareabed A, Nematnejad M, Yaghini J, Sadeghi SM. A comparative evaluation of the antimicrobial effect of chamomile, Aloe vera-green tea, and chlorhexidine mouthwashes on some oral bacterial species. *Dental Research Journal.* 2023 Jun;20(1):70. Doi: 10.4103/1735-3327.379627.
16. Gedam KY, Katre AN. Efficacy of Probiotic, Chlorhexidine, and Sodium Fluoride Mouthrinses on Mutans Streptococci in 8- to 12-Year-Old Children: A Crossover Randomized Trial. *Lifestyle Genomics.* 2022 Jan;15(1):35–44. Doi: 10.1159/000519916
17. Shah S, Bargale S, Dave BH, Deshpande A, Kariya PB, Karri A. Comparison of Antimicrobial Efficacy of (between) 0.2% Chlorhexidine and Herbal Mouthwash on Salivary Streptococcus mutans: A Randomized Controlled Pilot Study. *Contemporary Clinical Dentistry.* 2018 Sep;9(3):440. Doi: 10.4103/ccd.ccd\_264\_18
18. Padiyar B, Marwah N, Gupta S, Padiyar N. Comparative Evaluation of Effects of Triphala, Garlic Extracts, and Chlorhexidine Mouthwashes on Salivary Streptococcus mutans Counts and Oral Hygiene Status. *Int J Clin Pediatr Dent.* 2018 Aug;11(4):299–306. Doi: 10.5005/jp-journals-10005-1530
19. Somaraj V, Shenoy RP, Shenoy Panchmal G, Kumar V, Jodalli PS, Sonde L. Effect of Herbal and Fluoride Mouth Rinses on Streptococcus mutans and Dental Caries among 12–15-Year-Old School Children: A Randomized Controlled Trial. *International Journal of Dentistry.* 2017 Mar;2017(1). Doi: 10.1155/2017/5654373.

20. Vangipuram S, Jha A, Bhashyam M. Comparative efficacy of aloe vera mouthwash and chlorhexidine on periodontal health: A randomized controlled trial. *J Clin Exp Dent*. 2016 Oct;8(4):442-447. Doi: 10.4317/jced.53033.
21. Jain I, Jain P. Comparative evaluation of antimicrobial efficacy of three different formulations of mouth rinses with multi-herbal mouth rinse. *Journal of Indian Society of Pedodontics and Preventive Dentistry*. 2016 Oct-Dec;34(4):315-323. Doi: 10.4103/0970-4388.191409.
22. Mahajan R, Khinda P, Gill A, Kaur J, Saravanan S, Sahewal A, et al. Comparison of Efficacy of 0.2% Chlorhexidine Gluconate and Herbal Mouthrinses on Dental Plaque: An in vitro Comparative Study. *European Journal of Medicinal Plants*. 2016 Jan;13(2):1-11. Doi: 10.9734/EJMP/2016/23318.
23. Thomas A, Thakur S, Mhambrey S. Comparison of the antimicrobial efficacy of chlorhexidine, chlorhexidine, sodium fluoride, fluoride with essential oils, alum, green tea, and garlic with lime mouth rinses on cariogenic microbes. *Journal of International Society of Preventive and Community Dentistry*. 2015 Jul-Aug; 5(4): 302-308. Doi: 10.4103/2231-0762.161759
24. Bhat N, Mitra R, Oza S, Mantu VK, Bishnoi S, Gohil M, et al. The antiplaque effect of herbal mouthwash in comparison to chlorhexidine in human gingival disease: a randomized placebo controlled clinical trial. *Journal of Complementary and Integrative Medicine*. 2014 Jun;11(2):129-37. Doi: 10.1515/jcim-2014-0002.
25. Mohamed A, Wafaie K, Mohammed H, Mohamed AMA, Xinrui W, Vandevska-Radunovic V, et al. Effect of chlorhexidine mouthwash on gingival health around orthodontic miniscrew implants: A pilot placebo-controlled randomized trial. *Orthodontics & Craniofacial Research*. 2023 Jun;26(2):163-170. Doi: 10.1111/ocr.12596
26. Katz J, Garcia IA. The use of chlorhexidine mouthwash and diagnosis primary hypertension in a large hospital cohort. *EBSCOhost*. 2025 Feb;56(2):138. Doi: 10.3290/j.qi.b5872795.
27. Polizzi E, Tetè G, Bova F, Pantaleo G, Gastaldi G, Capparè P, et al. Antibacterial properties and side effects of chlorhexidine-based mouthwashes. A prospective, randomized clinical study. *Journal of Osseointegration*. 2020 Apr;12(1):2-7. Doi: 10.23805/JO.2019.12.01.20.
28. Calvo-Guirado JL, Fernández Domínguez M, Aragonese JM, Martínez González JM, Fernández-Boderau E, Garcés-Villalá MA, et al. Evaluation of new Seawater-based Mouth Rinse Versus Chlorhexidine 0.2% Reducing Plaque and Gingivitis Indexes. A Randomized Controlled Pilot Study. *Applied Sciences*. 2020 Jan;10(3):982. Doi: 10.3390/app10030982
29. Janket SJ, Lee C, Surakka M, Jangam TG, Van Dyke TE, Baird AE, et al. Oral hygiene, mouthwash usage and cardiovascular mortality during 18.8 years of follow-up. *Br Dent J*. 2023 Feb;1-6. Doi: 10.1038/s41415-023-5507-4
30. Di Lodovico S, Dotta TC, Cellini L, Iezzi G, D'Ercole S, Petrini M. The Antibacterial and Antifungal Capacity of Eight Commercially Available Types of Mouthwash against Oral Microorganisms: An In Vitro Study. *Antibiotics*. 2023 Apr;12(4):675. Doi: 10.3390/antibiotics12040675.
31. Ozmeric N, Enver A, Isler SC, Gökmenoğlu C, Topaloğlu M, Selamet H, et al. Evaluating the effects of chlorhexidine and vitamin c mouthwash on oral health in non-surgical periodontal therapy: a randomized controlled clinical trial. *Sci Rep*. 2025 Jan 29;15(1): 3703. Doi: 10.1038/s41598-025-88100-6
32. Bhor K, Shetty V, Garcha V, Ambildhok K, Vinay V, Nimbalkar G. Effect of 0.4% Triphala and 0.12% chlorhexidine mouthwash on dental plaque, gingival inflammation, and microbial growth in 14-15-year-old school children: A randomized control trial. *Journal of Indian Society of Periodontology*. 2021 Nov-Dec; 25(6):518-524. Doi: 10.4103/jisp.jisp\_338\_20.
33. Khobragade VR, Vishwakarma PY, Dodamani AS, Jain VM, Mali GV, Kshirsagar MM. Comparative Evaluation of Indigenous Herbal Mouthwash with 0.2% Chlorhexidine Gluconate Mouthwash in Prevention of Plaque and Gingivitis: A Clinico-Microbiological Study. *Journal of Indian Association of Public Health Dentistry*. 2020 Jun;18(2):111-117. Doi: 10.4103/jiaphd.jiaphd\_132\_19.
34. Ellis S. The effect of chlorhexidine mouthwash vs propolis mouthwash on the nitrate-reducing activity of oral bacteria and vascular control in healthy individuals. *The Plymouth Student Scientist*. 2021 Jul;14(1):1-13. Doi: 10.24382/7hby-ke07
35. Vinuesa Aumedes T, Hermida-Cabrera P, Aguilera FR, Vivancos-Cuadras F, Ferrá-Domingo L, Torres-Lagares D, et al. Evaluation of a new mouthwash formulated with Chlorhexidine and Cymenolafter a scaling and root planing treatment in grade I and II periodontal patients. *Articles publicats en revistes*. 2024 Nov;29(6):843-849. Doi: 10.4317/medoral.26818.
36. Oo MMT, Oo PH, Saddki N. Efficacy of 0.05% cetylpyridinium chloride mouthwash as an adjunct to toothbrushing compared with 0.12% chlorhexidine gluconate mouthwash in reducing dental plaque and gingival inflammation: A randomized control trial. *International Journal of Dental Hygiene*. 2023 Feb;21(1):195-202. Doi: 10.1111/idh.12614.
37. Chen W, Chen J, Bai D, Wang P, Shu R. Effects of clear aligners and traditional removable appliances on oral microbiome in mixed dentition: a comparative study. *BMC Oral Health*. 2024 Oct;24(1):1276. Doi: 10.1186/s12903-024-05063-2.