The Effect of Mouthwash in Oral Microbiome: A Literature Review

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Abstract:
The oral microbiome contains over seven hundred bacterial species as well as fungi, viruses, archaea, and protozoa. The complex equilibrium between resident species in the oral cavity is responsible for the maintenance of a healthy state or a state associated with disease. Mouthwash has also been shown to reduce plaque and gingivitis. The purpose of this article is to update our current knowledge of the effect of mouthwash in oral microbiome and to discuss the impact of mouthwash on changes in the oral microbiome.

The article was conducted of two databases and were limited to period January 2019 to May 2024 with combination of the following keywords: “oral microbiome” associated by the Boolean operator AND. The results obtained were 11 articles found at the beginning of the search in both databases, and the 6 full text articles were selected for further review and discussion. Commensal, gingivitis and halitosis associated species were significantly reduced by twice daily use of LCM (Listerine Cool Mint) or ACPM (alcohol-containing prototype mouthrinse). The AFPM (alcohol-free prototype mouthrinse) group showed no statistically significant reductions. Its microbiological action is not due to selective killing of pathogenic bacteria but rather via a reset mechanism, in which the plaque microbiome composition is shifted to a healthier state after repeated use. CHX (Chlorhexidine) led to an increase in the abundance of some genera such as Neisseria, Streptococcus and Granulicatella, and lowered the abundance of Actinomyces, but did not affect the abundance of Veillonella. The effect of mouthwash in oral microbiome are short-term and long-term impacts on supragingival plaque microbiome, return from dysbiosis to healthier levels, increasing abundance of common oral opportunistic bacteria, reduce the oral pathogenic microbial load in healthy subjects.

Keywords: effect, oral microbiome, mouthwash.
Introduction

The oral microbiome contains over seven hundred bacterial species as well as fungi, viruses, archaea, and protozoa (Sultan et al. 2018). There are several factors influencing the microbiota composition such as age, pregnancy, dietary habits like probiotic, (Balafif et al. 2023b) alcohol, tabagism, social status, medications (antibiotics), toxic substances, and genetic predisposition (Li et al. 2022). The complex equilibrium between resident species in the oral cavity is responsible for the maintenance of a healthy state (in symbiosis) or a state associated with disease (in dysbiosis) (Kilian et al. 2016).

Antimicrobial activity of essential oils mouthwash has also been documented. The popular mouthwash brand Listerine® (LIS), which contains four plant-derived essential oils (eucalyptol, menthol, methyl salicylate, thymol), has also been shown to reduce plaque and gingivitis (Chouhan, Sharma, and Guleria 2017).

The purpose of this article is to update our current knowledge of the effect of mouthwash in oral microbiome and to discuss the impact of mouthwash on changes in the oral microbiome.

Methods

This study is a descriptive and qualitative literature review. The literature review text was structured according to the PRISMA items. The article was conducted of two databases and were limited to period January 2019 to Mei 2024, which had their full texts published in English with combination of the following keywords “effect”, “mouthwash” or “mouthrinse”, “oral microbiome” associated by the Boolean operator AND. Duplicate articles, which were in different databases, were excluded from the review. Articles that appeared to meet the inclusion criteria, as well as articles that lacked information in their abstracts, were selected for full reading in phase 2, in order to determine the work eligibility. A supplementary article was included after checking the reference lists.

Results

The article selection 11 articles were selected from Pubmed (3 articles) and Google Scholar (8 articles). After reviewing titles and abstracts, 10 articles were excluded as they were not related to the review topic. A total of six articles were selected as they were related with the topic.

Short-Term and Long-Term Impacts on Supragingival Plaque Microbiome

Commensals, gingivitis and halitosis associated species were significantly reduced by twice daily use of LCM (Listerine Cool Mint) or ACPM (alcohol-containing prototype mouthrinse) compared with the HA (hydroalcohol) group after 1, 4, and 6 weeks. The AFPM (alcohol-free prototype mouthrinse) group showed no statistically significant reductions. Furthermore, no significant differences were observed for acidogenic species between the mouthrinse treatments and the HA negative control group, reflecting the clinical trial eligibility criteria that excluded subjects at risk of caries development.(Min et al. 2024)

Return from Dysbiosis to Healthier Levels

The microbiota of subjects with gingivitis were distinct from those of the healthy reference at baseline. Differential abundance testing revealed that several species were significantly more abundant in subjects with gingivitis, including commensals and key pathogens associated with gingivitis, such as Fusobacterium nucleatum, A. actinomycetemcomitans, and Prevotella species. Comparisons at post-baseline visits demonstrated changes in the microbiome composition of subjects with gingivitis in the LCM (Listerine Cool Mint) and ACPM (alcohol-containing prototype mouthrinse) groups, resembling the healthy cohorts at baseline, whereas the microbiome composition of the AFPM (alcohol-free prototype mouthrinse) and HA (hydroalcohol) groups resembled that of subjects with gingivitis at baseline. Further examination of individual bacteria abundances showed that many oral bacterial species were significantly reduced to levels comparable or lower than those found in healthy cohorts (Min et al. 2024).
Efficacy of Listerine for reducing plaque and gingivitis. Its microbiological action is not due to selective killing of pathogenic bacteria but rather via a reset mechanism, in which the plaque microbiome composition is shifted to a healthier state after repeated use (Min et al. 2024).

**Increasing Abundance of Common Oral Opportunistic Bacteria**

*Streptococcus* was the most abundant genus in most samples 61.7%, followed by *Prevotella* 13.5% and *Veillonella* 10%. The composition of the oral microbiome at the genus level (beta diversity) was significantly different after 3 months of Listerine or placebo use.*Fusobacterium nucleatum* and *Streptococcus anginosus* were significantly more abundant after Listerine use compared to baseline (Laumen et al. 2024).

Listerine use was associated with an increased abundance of common oral opportunistic bacteria previously reported to be enriched in periodontal diseases, oesophageal and colorectal cancer, and systemic diseases. These findings suggest that the regular use of Listerine mouthwash should be carefully considered (Laumen et al. 2024).

The impact of 7-day use of CHX on the oral microbiome showed that CHX led to an increase in the abundance of some genera such as *Neisseria*, *Streptococcus* and *Granulicatella*, and lowered the abundance of *Actinomyces*, but did not affect the abundance of *Veillonella*. However, it remains difficult to determine whether these microbial changes suggest a shift towards a healthy oral environment, or whether they may increase the risk of oral disease, as both increases and decreases in the bacteria associated with caries and periodontal disease have been reported (Bescos et al. 2020).

**Reduce the Oral Pathogenic Microbial Load in Healthy Subjects**

The DMW (dextranase-containing mouthwash) formulation I and DNMW (dextranase-and-nisin-containing mouthwash) formulation II reduced the PI (plaque index) without affecting the PBD (probing depth), GI (gingival index), and BOP (bleeding on probing) scores in healthy subjects. The use of DNMW for thirty days could significantly reduce the oral pathogenic microbial load in healthy subjects. In detail, DNMW has a more significant impact on reducing oral pathogenic genus *Pseudomonas*, *Veillonella*, *Stenotrophomonas*, *Achromobacter*, *Serratia*, *Brevundimonas*, *Klebsiella*, *Agrobacterium*, *Lactobacillus*, and *Trepnoma*, which may cause oral diseases. The DMW and DNMW showed a significant reduction in the biofilm of *S. mutans*. The DMW and DNMW mouthwashes could be an adjuvant to treat and manage oral diseases. However, some of the pathogens’ loads also increased after the use of mouthwashes. Thus, the results need further confirmation by experimenting with more study subjects (Chaiyasut et al. 2022).

**Discussion**

Chlorhexidine is an antimicrobial agent that is commonly used in the preoperative preparation of skin to prevent postoperative infections, and dental plaque prevention (Balafif et al. 2023a). There is a decreased relative abundance in several bacterial activities after gargling Chlorhexidine (CHX), e.g., bacterial chemotaxis, flagellar assembly, and lipopolysaccharide (LPS) biosynthesis. CHX may prefer to target gram-negative bacteria because LPS is a major component of their outer membrane. CHX binds to LPS and suppresses the LPS-induced inflammation after the bacteria are destroyed. CHX has been shown to reduce volatile sulfur compounds more effectively than essential oil mouthwash, but the pathway was not consistently different after gargling CHX.(Liu et al. 2023).

The significant decrease in the abundance of Bacteroidetes after using CHX. This was the second most abundant phyla in the oral cavity and some genera from this phyla such as *Veillonella* has been shown to be important in maintaining the acid/base conditions in the mouth. Overall, these findings indicate that CHX promotes acidification of saliva by changing the ratio abundance of different families of bacteria that are essential to maintain the acid/base conditions in the mouth of healthy people (Brooks 2018; Bescos et al. 2020).
Preference of Listerine on target bacteria is also hinted in the pathway analysis. After gargling Listerine, the relative abundance of “vitamin B6 metabolism” decreased. Many bacteria in the Bacteroidetes phylum possess a vitamin B6 biosynthesis pathway, while most Firmicutes lack such the pathway. After gargling Listerine, Bacteroidetes decreased while Firmicutes increased, which could explain the decreased “vitamin B6 metabolism”. We also observed the increase of “sulfur metabolism” after gargling. Listerine seems reasonable because volatile sulfur compounds produced by bacteria are the source of oral malodor, and Listerine may target those bacteria (Liu et al. 2023).

### Conclusion

The effect of mouthwash in oral microbiome are short-term and long-term impacts on supragingival plaque microbiome, return from dysbiosis to healthier levels, increasing abundance of common oral opportunistic bacteria, reduce the oral pathogenic microbial load in healthy subjects.

### Conflict of interests

No conflict of interest.

### References


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