Cantaurus, Vol. 28, 25-27, May 2020 © McPherson College Department of Natural Science

Activity of mouthwash against *Streptococcus* species in oral cavity

Angel Salmeron Rattana

ABSTRACT

Mouthwash is one of the methods in maintaining a healthy oral cavity. Mouthwashes can be either cosmetic or therapeutic. Therapeutic formulas may control plaque and tooth decay in addition to fighting bad breath. The bacterial control focused on in this study is *Streptococcus salivarius* and *Streptococcus mutans*. The bacteria will be grown on dextrose agar or 5% sheep blood agar that will be rinsed by alcohol, no-alcohol, and hydrogen peroxide mouthwash. The growth of bacterial colony should determine relative effectiveness of each mouthwash tested.

Keywords: *Streptococcus salivarius, Streptococcus mutans,*

INTRODUCTION

The human oral cavity contains over 6 billion bacteria, including 700 different species. (Jorn *et al.*, 2005) The population growth of specific bacteria in the oral cavity produce a biofilm covering the surface of the tooth dentin. The development of a tooth biofilm may lead to dental carries and halitosis and will require interventive hygiene. In the mid 1800's, before oral hygiene was popularized, the drinking of coffee and tea led to discoloration of the teeth. In 1873 a popularized toothpaste (Colgate) was marketed and in 1879 Listerine mouthwash was developed promoting mass production of oral hygiene products to keep the oral cavity maintained. Dietary influences affect the amount of sugars available to the microbiome in their production of plaque. This varies in consumption of simple sugars and acidic liquids. Maintaining a healthy oral cavity begins with proper dietary nutrition and daily hygiene practices such as flossing, brushing teeth, and mouthwashes. (Elamin, 2018) Therapeutic mouthwashes are often labeled with a statement of their effectiveness for eliminating 99.99% of oral bacteria. Consumers are often inundated with information on mouthwash advertisements in understanding efficacy for general purposes of combatting halitosis versus controlling plaque build up. Therapeutic mouthwashes can be categorized into three groups: Antiseptic, plaque inhibiting, and preventive, which is the most commonly used mouthwash due to the amount of fluoride. Of these three type of therapeutic mouthwashes, this research presented in this paper focused on which types actually destroy the most bacteria in the oral cavity.

Many case studies focus on *Streptococcus mutans*, which is the main bacterium that causes dental carries. (Forssten et al., 2010) *Streptococcus salivarius* is a beneficial bacterium found in the saliva of the oral cavity. Saliva helps with the remineralization process of dentin by bringing in more calcium, phosphate, and fluoride ions. Mouthwash efficacy has often been tested against the streptococcus family of bacteria. (Salehi, 2006; Syahdiana, 2018)

Mouthwashes have different elements in them, such as containing chlorhexidine, persica, alcohol. Mouthwashes vary in their chemical efficacy concentration on decreasing bacterial populations. Chlorhexidine is shown to have the most effect in the amount of bacteria after use. (Haerian-Ardakani, 2015) The active ingredient chlorhexidine has the greatest impact on bacterial reduction. However, chlorhexidine mouthwashes have side effects such as leaving a bad taste, tooth discoloration, and a burning sensation. (Salehi, 2006) Hydrogen peroxide can be used as an oral rinse in its diluted form as long as it is not ingested. The 3% dilution form of hydrogen peroxide is shown to be effective against certain bacteria.. Giving a general idea of how much bacteria is actually being reduced by every single mouthwash could help not only myself, but other buyers to decide on which mouthwashes to use. Many articles also have different types of procedures that are used to obtain their results and how to obtain their bacteria. One study used a tiny rubber-band to put in-between the molar teeth and upper canine to extract the bacteria. (Salehi, 2006) Another study cultivated the bacteria *Streptococcus mutans* in a medium and then measured the diameter of the colonies that had been cultivated on an agar medium. (Cardoso, 2011) The overall idea of this experiment is to implement as many mouthwashes as possible against 2 types of bacteria. The bacteria that will be tested is *S.mutans* and *S.salivarius*.

MATERIALS AND METHODS

Lyophilized cultures of *S.mutans* and *S.salivarius* were rehydrated according to the manufacterer's directions (Carolina Biological Supplies and Wards), and incubated at 37°C for 48-72 hours. After the incubation period, a 24-hour culture was created by taking 10 uL of the bacteria and placing it in 10mL nutrient broth. On the day of the experiment, the tryptic

soy agar plates with 5% sheep's blood was labeled. Each plate was labeled with the corresponding mouthwash and bacteria. With a sterile volumetric pipette, 0.1mL of the bacteria was spread onto the agar. The plate was rotated in a clockwise fashion with continuous back and forth motion, so that the entire plate is covered with bacteria. Five milliliters of the corresponding mouthwash or deionized water (DI control group) was added to the agar plates and swirled in a circular motion to cover the entire plate for 60 seconds. Immediately after, the liquid was discarded. Sealed purchased mouthwashes were used for their sterility, while the deionized (DI) water was sterilized prior to use. Procedures were systematic for ten replications in each mouthwash and control group. Processed agar plates were inverted in an incubator at 37 C for 5 days. The laminar flow hood and all workspaces were cleaned before and after use with 70% ethanol.

RESULTS

After allowing the bacteria to incubate for 5 days the results were observed. The presence of bacterial colonies were enumerated on each plate that showed a sign of growth. The bacterial growth of *S.mutans* was inconclusive. This was the second attempt of trying to grow this bacterial strain, which did not work for either attempts; 0 out of 10 plates grew for the no-alcohol mouthwash. Alcohol mouthwash had 2 out of 10 plates that exhibited growth. Hydrogen peroxide mouthwash inhibited bacterial growth on all plates. The control group, water had 4 out of 10 plates that exhibited growth.

Table 1 Cultures of *S. mutans* and *S. salivarius* were each inoculated onto 10 plates of dextrose agar and then rinsed off with the corresponding mouthwash or water.

Mouthwash	*S. mutans*	*S. salivarius*
No-Alcohol (Crest Pro)	0	0
Alcohol (Listerine)	0	2
Hydrogen Peroxide (Crest 3d)	0	0
Water(Control)	0	4

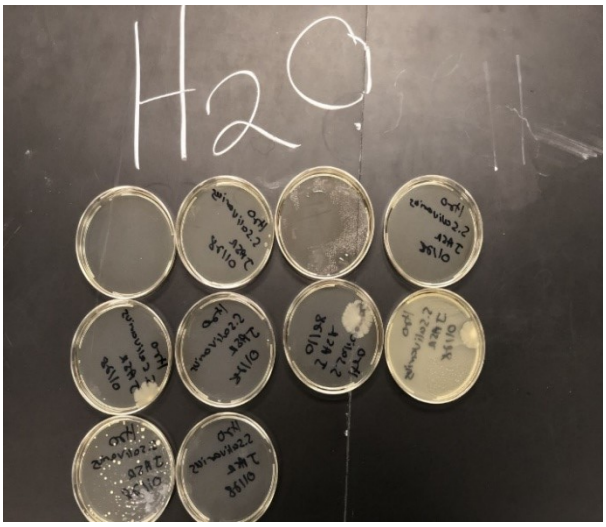

Figure 1 *S. salivarius* growth on dextrose agar after a 5-day incubation period from the control group

Figure 2 *S. salivarius* growth on dextrose agar after a 5-day incubation period from the alcohol mouthwash

DISCUSSION

Based on the results, the mouthwashes tested did not eliminate 99.99% of *S.salivarius*. This study only focused on one bacterium whereas the oral cavity is a community of bacteria interacting and may not respond in a similar fashion. Another limitation that should also be accounted for is the fact that there was only a sample size of ten that were computed for each mouthwash. Manufacturers also do not show the amount of samples that they had used to give their results. Manufactures should base their results on continuous studies. This study does not account for every mouthwash and brands of mouthwash.

S.mutans was not culturable in this study, which may have been impacted by the procedure of rehydrating the lyophilized bacteria.. Another observation that can be made is the fact that the water that was autoclaved only had 4 out of the 10 plates with bacterial growth. This could be a possibility that rinsing the oral cavity could physically remove bacteria and not from antibacterial properties. The plates were immediately rinsed after being inoculated, which did not allow the bacterial broth to set onto the agar properly. A way to avoid this is to allow the plates to set for an hour after inoculation.

ACKNOWLEDGEMENTS

Jonathan Frye, Karrie Rathbone, McPherson College Natural Science Department

LITERATURE CITED

Bahlouli, S, Z Aghazadeh, M Aghazadeh, S Shojani, H Kafil. 2018. Determining the Antibacterial Activity of Chlorhexidine Mouthwashes with and without Alcohol against Common Oral Pathogens. Journal of Advanced Oral Research 9(1):1-5.

Cardoso, T, A Carvalho, M Beletti, M Napimoga and G Thedei. 2011. Metabolic activity of *Streptococcus mutans* biofilms after treatment with different mouthwash formulations. Braz J Oral Sci. 10(1):74-78.

Elamin, A, M Garemo and A Gardner. 2018. Dental caries and their association with socioeconomic characteristics, oral hygiene practices and eating habits among preschool children in Abu Dhabi, United Arab Emirates — the NOPLAS project. BMC Oral Health 18:104.

Garcia, S, S, M.S. Blackledge, S. Michalek, L. Su, T, Ptacek, P. Eipers, C. Morrow, E.J. Lefkowitz, C. Melander and H, Wu. 2017. Targeting of *Streptococcus mutans* Biofilms by a Novel Small Molecule Prevents Dental Caries and Preserves the Oral Microbiome. J Dent Res. 97(7): 807-814.

Groessner, B, M Hanning, A Duck, M Griepentrog and DF Wenderoth. 2004. Do different implant surfaces exposed in the oral cavity of humans show different biofilm compositions and activities? Eur J Oral Sci 112:516-522.

Haerian-Ardakani, A, M Rezaei, M Talebi-Ardakani, N Keshavarz Valian, R Amid, M Memandi, A Esmailnejad, A Ariankia. 2015. Comparison of Antimicrobial Effects of Three Different Mouthwashes. Iran Journal of Public Health 44(7): 997-1003.

Jorn, A, B Paster, L Stokes, I Olsen, F Dewhirst. 2005. Defining the Normal Bacterial Flora of the Oral Cavity. Journal of Clinical Microbiology, pp. 5721-5732.

Jothika, M, P Pranav and B Someshwar. 2015. Effectiveness of probiotic, chlorhexidine and fluoride mouthwash against *Streptococcus mutans* – Randomized, single-blind, *in vivo* study. J Int Soc Prev Community Dent 5(Suppl 1):S44-S48.

Salehi, P., and Sh. Momeni Danaie. 2006. Comparison of the antibacterial effects of persica mouthwash with chlorhexidine on Streptococcus mutans in orthodontic patients. DARU Journal of Pharmaceutical Sciences 14(4):178-182.

Syahdiana, W, D Suryanto, and Yurnaliza. 2018. Antibacterial activity of cinnamon ethanol extract (Cinnamomum burmanii) and its application as a mouthwash to inhibit Streptococcus growth. IOP Conference Series: Earth Environment Science 130: 012049