The Short Term and Long Term Effectiveness of Listerine® Antiseptic Mouthwash in reducing Streptococcus mutans

Introduction

Extensive research has been performed on the effectiveness of oral mouthwashes in inhibiting the development of microorganisms in the oral cavity. Listerine antiseptic mouthwash has been tested in several laboratories on its ability to prevent plaque formation (Kato et al. 1990). Previous evidence indicates that short-term usage of Listerine has the potential to inhibit the development of plaque and likewise reduce the effects of gingivitis (Mankodi et al. 1986). It has been hypothesized that frequent usage of antiseptic mouthwashes may lead to a succession of opportunistic microorganisms due to increased resistance (Jenkinson 1995). Studies, however, have shown that there is no significant shift in microbial composition within a six-month usage period (Minah et al., 1988). Our lab experiment was designed to deduce both short-term and long-term effectiveness of Listerine antiseptic mouthwash in preventing the formation of bacterial Streptococcus mutans. Therefore, if Listerine decreases the formation of S. mutans found in the mouth, then I predict that the number of bacterial colonies will be lower after rinsing with Listerine than before. Likewise if Listerine is effective in killing bacteria over a long-term period, then we predict that resistance is not a factor and no significant increase in S. mutans will be observed.

Methods and Materials

The Streptococcus mutans in this experiment were grown on Mitas Salvarius Bacitracin Agar. This particular type of agar consists of 90 g/L of Mitas Salvarius agar, 150 g/L sucrose, 600 units/ml of sterilized Bacitracin Filter, and a Chapman tellurite solution. The bottom of the agar plates were divided into two equal halves; one for the control, in which the subject rinsed with water, and the other for the treatment, in which the subject rinsed with Listerine. After the bacterial samples of both the control and the variable were properly positioned, the lids were placed on the agar dishes and put into a Thelco model four incubator for seven consecutive days in an environment of high
carbon dioxide and a constant temperature of 28°C.

The first part of the experiment consisted of developing a control in order to provide an accurate means of comparison and analysis of the measured variable. The selected individual rinsed with 5ml of water for a duration of thirty seconds. Immediately after, the individual chewed on parafilm for 1 minute and then used a tongue depressor to absorb saliva. The tongue depressor was placed on the designated “control” half of the agar. In the second part of the experiment, Listerine® was used to deduce its effectiveness against S. mutans. The same methods were followed as above except the sample was placed on the “treatment” half of the agar plate.

After both the control and the variable parts of the experiment were performed and were present on the agar dish, the dish was then placed inside an incubator for a period of seven days where the bacteria were allowed to grow and reproduce in an environment of 28°C. The plates were examined under dissecting scopes and the number of black, raised colonies of S. mutans were counted. I calculated means and performed statistical analysis using Microsoft Excel.

Results

Individuals with no prior usage of Listerine Antiseptic showed a significant (p=1.61E-12) decrease in the number of S. mutans colonies after treatment of Listerine was administered (Fig.1). In other words the number of bacterial colonies in the control sample was substantially greater than in the treatment sample. Listerine, likewise, had a significant impact (p=7.49E-6) on the number of S. mutans colonies found in individuals who had previously used Listerine. The number of bacterial colonies was lower in the treated sample than in the control sample. However, there were no significant (p=0.23) changes in the abundance of S. mutans colonies between the two control samples, proving Listerine to be ineffective over a longer period of time.

The percent reduction (difference in the number of bacterial colonies in the control and the treatment sample divided by the control sample) in S. mutans colonies in the non-users compared to Listerine users, proved to be insignificant (p=.426) (Fig. 2). Figure 3 shows that time had little or no influence in determining the abundance of bacterial colonies (R^2=.0270).
Discussion

Results from the lab experiment indicate that Listerine decreased the number of \textit{S. mutans} colonies found in the mouth. While Listerine was effective over a short-term period of time, the comparison between the two controls is insignificant, suggested that Listerine was ineffective over an extended time interval. The ineffectiveness of Listerine over a long term period of time may be an indication of an increase in bacterial resistance or that Listerine users were not rinsing with enough frequency to detect a significant difference. Though we do not know the frequency in which individuals used Listerine, we detected resistance by comparing the users and non-users of Listerine to the percent reduction of \textit{S. mutans} colonies. The rate that bacterial colonies rebounded after Listerine usage was not determined by the time since last usage. The overall results supported our short-term prediction in which Listerine decreased the number of \textit{S. mutans} colonies after usage. The results likewise show that resistance was not a factor in determining the number of \textit{S. mutans} colonies but, unlike our long-term prediction, was independent of the long-term effectiveness of Listerine Antiseptic. In fact, Listerine was ineffective over a long period of time.

The frequency in which users actually used Listerine was not taken into account and thus, may have resulted in possible error by introducing an unaccounted variable into the experiment. In addition, the temperature in which the bacterium was incubated may have had an effect on the number of bacterial colonies. The bacteria in our lab were incubated at a temperature of 28°C when, according to the lab directions, they should have been exposed to a 35°C environment. This may have skewed the overall results.

The results of this study agreed with Kato et al (1990) in which their findings likewise found that Listerine significantly decreased plaque formation caused by various microorganisms. Our experiment also concurred with the findings of Minah et al (1988), demonstrating that long term usage of antiseptic mouthwash does not cause any significant shift in bacterial emergence. This conclusion, however, is in direct conflict with the experiment of Jenkinson (1996) who stated that microbial cells have indeed acquired resistance.

The methodology carried out in our experiment differed from similar studies in several ways. Unlike our study in which each person represented both the control and the
treatment part of the experiment, other studies separated individuals into distinct control or treatment groups (Mankodi et al. 1986). Though our experiment tested long-term effectiveness of Listerine over a period of hours, other studies tested the longevity of Listerine over a period of months, leading to greater accuracy. Yet other differences in comparing our experiment with those of others include the type of species being tested, and the collection format used to obtain the microorganisms. Several studies tested the effectiveness of Listerine against various species of bacteria while our experiment targeted only *S. mutans* bacteria (Jenkinson 1996). Testing the effect of Listerine against other forms of bacteria may yield different results.

**Literature Cited**


Figures

![Bar chart showing the control and treatment of non-users and users of Listerine vs. the number of Streptococcus mutans colonies.](image)

**Figure 1:** Control and treatment of non-users and users of Listerine vs. the number of *Streptococcus mutans* colonies.
Figure 2: Non-users and users of Listerine compared to the percent reduction of *Streptococcus mutans* colonies.
**Figure 3:** Analysis of the time (in hours) since last use of Listerine vs. the number of *Streptococcus mutans* colonies. 

$R^2 = 0.027$