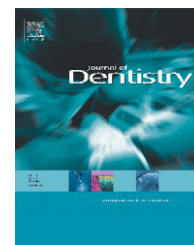


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Germicidal activity of antimicrobials and VIOLight[®] Personal Travel Toothbrush Sanitizer: An *in vitro* study

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ABSTRACT

Objectives: The study evaluated the antibacterial effect of VIOLight[®] (VL) Personal Travel Toothbrush Sanitizer on biofilms after toothbrush exposure to human saliva compared to Listerine[®] Antiseptic (LA), 3% hydrogen peroxide (3%HP) and water.

Methods: Twenty toothbrush heads ($n = 5/\text{Gp}$) were immersed in saliva and to allow for bacterial growth and biofilm formation for 24 h. VL sanitizer and antiseptic(s) were used for 7 min; after treatment, brush heads were rinsed and placed into 10 mL of 2× AOAC Letheen Broth, sonicated and vortexed for 10 s. Tenfold serial dilutions were prepared and plated and incubated aerobically and anaerobically. Log₁₀ CFU/mL data were compared utilizing ANOVA ($p < 0.05$).

Results: Results showed 3%HP with significantly lower counts than LA, VL and control for aerobic and anaerobic bacteria. LA had significantly lower counts than VL and control for both types of bacteria and VIOLight[®] had significantly lower counts than the control for aerobic bacteria. 3%HP and LA were most effective in rapidly killing bacteria when compared to VIOLight[®].

Conclusions: Results showed that 3% hydrogen peroxide was most effective in reducing the numbers of both aerobic and anaerobic bacteria present on the toothbrush heads. Under the same test conditions, Listerine[®] Antiseptic was shown to be secondarily effective for the same bacteria while the VIOLight[®] unit was the least effective when compared to the other treatment groups.

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1. Introduction

The oral cavity hosts over 700 different bacterial species and represents an ideal habitat for the bacteria to grow and proliferate; this scenario increases the likelihood for developing periodontal diseases and tooth decay in humans. The toothbrush is still considered the most common and effective over-the-counter device that removes the oral bacterial biofilm and soft debris from the oral cavity, specifically from the surface of the teeth and tongue. However, in performing

such activity on daily basis the transfer or “retention” of bacteria on the toothbrush head is expected, therefore resulting in its oral bacterial contamination. In addition to oral microorganisms, other bacteria, fungi and yeast present in the surrounding external environments can also contribute to the bacterial load of toothbrushes.^{1,2} Bathrooms, the most common place in the house where toothbrushes are stored, seem to be a common place where fecal-oral spread of pathogenic viruses and bacteria can occur.^{3,4} In particular, isolation of enterobacteria from areas surrounding the toilet

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suggests the possibility that some aerosol contamination can occur by toilet flushing³; the minute drops of aerosol will then deposit on the toothbrush if placed in the proximity of the toilet. Furthermore, it has also been reported that toothbrushes kept in a humid environment like the bathroom retained up to 50 percent of Herpes Simplex virus Type I for about a week.⁵ Microorganisms present on a contaminated toothbrush can remain viable for a period ranging from 24 h to 7 days; this could imply that the daily use of an already contaminated toothbrush could contribute in spreading these microorganisms within the oral cavity or, when in direct contact, among other toothbrushes if stored in the same place.⁶ In fact, several studies have also shown that certain oral bacteria such as *Porphyromonas gingivalis* can possibly be transmitted between spouses⁷ and siblings as well as from parents to children.⁸ Although the friction resulting from brushing has been long recognized to be a stimulating factor for the oral epithelium,⁹ we still need to remember the potential transmissibility of bacteria into the hard and soft tissues of the oral cavity and overall body while using the toothbrush.

In a recent study, Mehta et al.¹⁰ evaluated the extent of bacterial contamination of used toothbrushes in a group of students living in similar environmental conditions and the efficacy of chlorhexidine, Listerine[®] and plastic cap in decontaminating the brushes ($n = 10$). The results of the study showed that seven of the 10 toothbrushes were contaminated and that chlorhexidine was more effective than Listerine[®]. However, Listerine[®] was more effective than the plastic caps which showed a greater growth of microorganisms like *Pseudomonas aeruginosa*, therefore remaining not an advisable practice for minimizing toothbrush contamination.

Spray antimicrobial solutions have also been tested to assess their efficacy level on toothbrushes decontamination. In an *in vivo* study, Sato et al.¹ evaluated the effectiveness of three antimicrobial-containing spray solutions (basic formulation spray, basic formulation with the addition of cetylpyridinium chloride (CPC) and a water control spray) in the decontamination of toothbrushes used for 1 week by participants. In this cross-over study, subjects ($n = 30$) received a brand new toothbrush for each period and were instructed to spray the solution 6 times after each brushing activity. At the end of week-use all toothbrushes were collected and evaluated for bacterial contamination. The results of this study showed that the highest microbial contamination was achieved with the control water spray; however, a statistically significant reduction in contamination level was observed when the other two spray formulations were used and compared to the control spray, thus suggesting that these two sprays are both feasible approaches for toothbrush decontamination.

Although chlorhexidine was considered as an alternative to one of the test products, preference was given to the over-the-counter products Listerine[®] Antiseptic and hydrogen peroxide because of their accessibility to the consumers.

The purpose of this study was to evaluate the antibacterial effect of VIOLight[®] Personal Travel Toothbrush Sanitizer in reducing bacteria found in human saliva artificially deposited on toothbrush heads, when compared to Listerine[®] Antiseptic, 3% hydrogen peroxide and water.

2. Materials and methods

A total of 20 new Oral-B manual toothbrush heads were used in this study. The toothbrush heads were first rinsed under sterile de-ionized water for 10 s prior to immersion in saliva. Saliva was collected from a number of healthy human volunteers with good general oral health. The saliva was collected in the early morning hours after subjects refrained from brushing from the night before. The saliva collected was unstimulated and it was collected from each individual in a sterile centrifuge tube (at least 5 mL from each volunteer). The saliva was then aseptically pooled and stored under refrigeration until it was used for testing (4–8 °C). The testing was conducted at ambient room temperature (19–24 °C). The toothbrush heads were immersed in the whole saliva for ~48 h at 37 ± 1 °C to allow for colonization and bacterial growth. The brush heads were rinsed under running tap water for 10 s prior to exposure to the ultraviolet (UV) toothbrush sanitizer unit or the antiseptic(s). The test articles were identified as follows: Listerine[®] Antiseptic Gold, 1 L, Lot#00737L, Aaron Brands 3% hydrogen peroxide solution (USP; First Aid Antiseptic, Lot#30030, three bottles of 473 mL each); and VIOLight[®] Personal Travel Toothbrush Sanitizer (Germicidal UV light), were used for testing. The toothbrush heads were exposed to the UV light treatment for 7 min as recommended by the manufacturer and they were treated with the antiseptics for the same exposure time as the VIOLight[®]. The antiseptic test articles were tested undiluted at ambient room temperature (20–25 °C).

After exposure to the test treatment, the toothbrush heads were rinsed under running tap water for 10 s, and the excess water was shaken off the brush head prior to neutralization, dilution and plating. The antibacterial activity was neutralized at the end of a specific exposure period. The neutralizer used for this study was 2× AOAC Letheen Broth. Neutralizer effectiveness for the test articles(s) was not determined as it is historically known to be effective.¹¹

A 20 s tap water rinse was then conducted on five brush heads to serve as the untreated numbers control. All tests involving the test articles and untreated control were conducted using five replicate brush heads ($n = 5$ /Group). For the antiseptic products, a 30 mL aliquot of the test article was placed in a sterile centrifuge tube at ambient room temperature. Toothbrush heads were then placed into the centrifuge tube containing the antiseptic; they were vortexed for 10 s and then allowed to remain in contact with the antiseptic for 7 min. Another 10 s vortexing occurred the last 10 s of the exposure period prior to aseptically removing the brush head for plating. At exactly 7 min, the treated brush heads were placed in 10 mL of 2× AOAC Letheen Broth (10^{-1} dilution). After the brush heads were exposed for 7 min in the UV toothbrush sanitizer unit, they were also placed in 10 mL of 2× AOAC Letheen Broth (10^{-1} dilution). The broth containing the brush head was sonicated for 10 s followed by vortexing for 10 s and further serially diluted in 0.1% Sterile Peptone Water. A 1 mL aliquot was removed from the tube (10^{-1} dilution) of 10 mL of 2× Letheen Broth and 10-fold serial dilutions (containing 9 mL of 0.1% Peptone Water) were prepared to 10^{-4} (plating 10^{-2} to 10^{-5}) for the test article(s) and to 10^{-7} (plating 10^{-5} to 10^{-8}) for the numbers control. The dilutions were plated in duplicate, for both aerobic and anaerobic

Table 1 – Calculation of % reduction.

$$\%Reduction = \frac{[NC(CFU/mL) - TA(CFU/mL)]}{NC(CFU/mL)} \times 100$$

NC: average numbers control population
TA: average test article population

incubation, by the Spread Plate Technique using Schaedler Blood Agar. The plates for aerobic incubation were incubated in an inverted position at 35 ± 2 °C for 48 ± 4 h. The plates for anaerobic incubation were incubated in an inverted position at 33–38 °C for 120 ± 4 h.

2.1. Statistical analysis

The number of colony forming units (CFU) recovered per sample dilution was tabulated and the total number of CFU/mL of sampling solution was calculated. Bacterial counts were also transformed into log₁₀ counts. Both the percent reduction in numbers and the log₁₀ reductions were reported for both aerobic and anaerobic bacteria for each of the three treatments. A percent reduction, as compared to the numbers control, was determined against the average result of the five replicates for the exposure period (Table 1). Log₁₀ reductions for each treatment were calculated by subtracting the average result of the five treatment replicates from the average result of the five control replicates. The treatments log₁₀ survivors were compared utilizing analysis of variance testing the hypothesis that all treatment means are equal. Follow up multiple comparison testing was conducted utilizing Tukey’s Studentized Range (HSD) Test. For this analysis the log₁₀ survivors of each individual treatment replicate were calculated vs the individual control replicates. All statistical tests of hypothesis will employ a level of significance of 0.05.

3. Results

Results of this study showed that treatment with hydrogen peroxide with 7 min of exposure was most effective in reducing the numbers of both aerobic (1.2 log reduction) and anaerobic (1.3 log reduction) bacteria present on the toothbrush heads (Fig. 1A and B) (*p* < 0.05 vs all other treatments based on analysis of variance tests). Under the same test conditions, Listerine® Antiseptic was shown to be secondarily

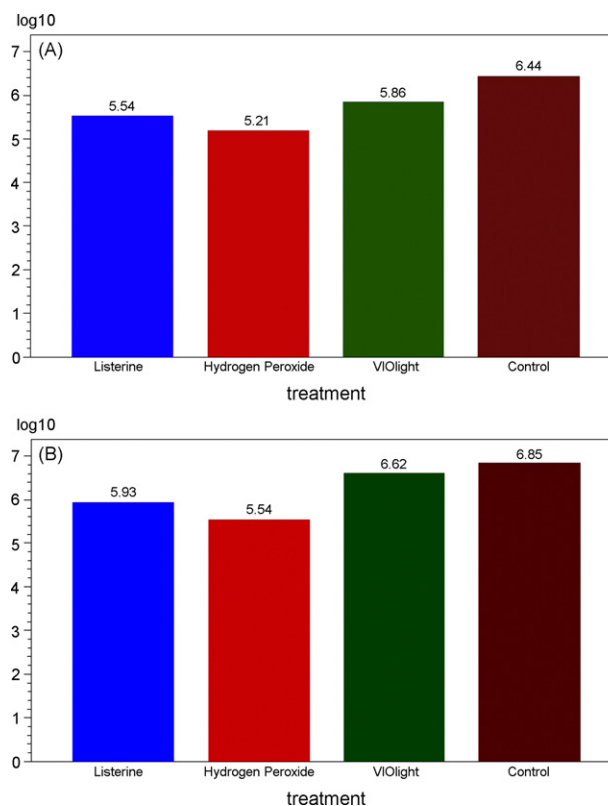


Fig. 1 – (A) Graph of the mean log₁₀ values—aerobic bacteria. (B) Graph of the mean log₁₀ values—anaerobic bacteria.

effective for both the aerobic and anaerobic bacteria (~0.9 log reduction) while the UV toothbrush sanitizer unit (VIOLight®) was shown to be least effective when compared to the other treatment groups. Mean CFU/mL and percent reduction in numbers of bacteria from the control and mean log₁₀ averages/mL and reductions from control are described in Table 2.

4. Discussion

The oral cavity presents a succession of different ecological situations with ages which also affects that composition of the

Table 2 – Mean CFU/mL (std), mean log₁₀ (std), mean log₁₀ average reductions from control, percent reduction.

Bacteria	Code	n	Mean CFU/ml	Mean log ₁₀ average	Mean log ₁₀ average reductions from control	Percent reduction
Aerobic ^a	Control	5	2.8E6	6.4420	–	–
	(1) Listerine®	5	3.8E5	5.5380	0.9040	86.43
	(2) 3%Hydrogen peroxide	5	1.6E5	5.2080	1.2340	94.28
	(3) VIOLight®	5	7.9E5	5.8560	0.5860	71.78
Anaerobic ^b	Control	5	7.9E6	6.8520	–	–
	(1) Listerine®	5	8.9E5	5.9280	0.9240	88.73
	(2) 3%Hydrogen peroxide	5	3.8E5	5.5420	1.3100	95.19
	(3) VIOLight®	5	4.3E6	6.6180	0.2340	45.57

^a Anova *p*-value comparing mean log₁₀: <0.0001. Significant comparisons: 2 vs 1, 3, control; 1 vs 3, control; 3 vs control.

^b Anova *p*-value comparing mean log₁₀: <0.0001. Significant comparisons: 2 vs 1, 3, control; 1 vs 3, control.

normal flora of the mouth.¹² Control of dental plaque is the most important factor for preventing major oral diseases such as gingivitis, periodontitis and caries. Although it has been widely demonstrated that over-the-counter products such as antimicrobial mouthwashes have potential for affecting the quality of colonizing bacteria,¹³ the use of mechanical methods such as toothbrushes and dental floss are still the primary avenues for disturbing the oral ecosystem thus reducing the quantity of pathogenic gram-negative and anaerobic bacteria.¹⁴ In the early 1920, Cobb reported that toothbrushes could have been the source responsible for repeated oral infections.¹⁵ Glass et al. have later demonstrated that contaminated toothbrushes could transmit viruses and bacteria that caused the onset of systemic, localized and oral inflammatory diseases and that regardless of the nature of the disease, individuals could have eliminated symptoms as well as the disease by just changing the toothbrush.^{16,17} A more recent study by Lock et al. demonstrated that toothbrushes used by Hepatitis-C patients were contaminated with HCV-RNS thus showing a theoretical risk of infection if these objects are shared with others.¹⁸ As of today, the Centers for Disease Control and Prevention are not aware of any adverse health effects directly related to toothbrush use. However, due to the limited research on this topic, the agency makes a clear recommendation that because the toothbrush becomes contaminated with bacteria, blood, saliva, oral debris and toothpaste is best practice to rinse the toothbrush thoroughly with tap water following brushing. Furthermore, the CDC states that to date there is insufficient clinical evidence showing that brushing with a contaminated toothbrush has led to recontamination of a one's mouth, oral infections, or other adverse effects.¹⁹

Therefore, even though there are no recent reports in the current literature that clearly demonstrate that recontamination of the human oral cavity occurs when a contaminated toothbrush is used for daily oral hygiene procedures, over the years different products and methodologies such as antimicrobial solutions, sprays and toothpastes,^{1,20,21} dishwasher washing²² and UV toothbrush sanitizing devices^{23,24} have been developed and evaluated for decreasing the oral and environmental bacterial load on toothbrush heads. However, as reported by Efstratiou et al., translocation of oral bacteria has been pointed out in past studies as a possible and viable method occurring with oral hygiene aids.²⁵ The same authors also evaluated the contamination and survival rate of periodontopathic and cariogenic species on new toothbrushes coated with antibacterial properties (triclosan coated filaments) in periodontitis patients and concluded that the coated toothbrush did not have a positive effect on bacterial contamination; however, when toothpaste was used a significant reduction of toothbrush contamination was observed.²⁵

There are several methods used to decrease the bacterial load of toothbrushes and as of today there is very literature available on toothbrush sanitizers providing specific health benefit. The ADA provides a list of recommendations for the dental patients on how to take proper care of their toothbrush and for those patients using toothbrush sanitizer it encourages them to select a toothbrush sanitizer that is cleared by the Food and Drug Administration (FDA).^{23,26} Furthermore, the ADA Council on Scientific Affairs' invites consumers and professionals to critically review the claims



Fig. 2 – Products tested: Listerine® Antiseptic, 3% hydrogen peroxide and VIOlight® toothbrush sanitizer.

made by manufactures of the toothbrush sanitizer which usually refer to sanitizing (not sterilizing) or reducing bacterial contamination on toothbrush.²⁷

In our study we have used two easily accessible over-the-counter antimicrobial products to evaluate their antibacterial effect on oral biofilm when compared to VIOlight®: 3% hydrogen peroxide and Listerine® Antiseptic (Fig. 2). Biofilms, a complex bacterial community occurring in a natural and artificial environments, are the results of bacterial adhesion and multiplication of cells on a surface resulting in a production of a matrix composed of extracellular polymeric substances (EPS) which not only provides protection to the biofilm by preventing access of biocides (i.e. hydrogen peroxide, tetracycline) but also by stimulating phenotypic variation and intercellular communication.^{28,29} Therefore, in order to kill and remove biofilm antimicrobials, such as oxidizing biocides like peroxides, must be able to penetrate the EPS. When hydrogen peroxide is applied to a surface, it reacts quickly and then breaks down into water and oxygen. At the same time, free oxygen radicals are released. These radicals create oxidation which is a chemical process in which oxygen combines with another substance to break down or change the function of the molecules. Thus through oxidation, the bacteria present on the surface quickly decomposes. On the other hand, the fixed-combination of essential oils (Listerine® Antiseptic) kills microorganisms by disrupting their cell walls and inhibiting their enzymatic activity thus preventing bacterial aggregation, slowing multiplication and extracting endotoxins. We can explain that the observed superiority of 3% hydrogen oxide in killing bacteria is due to its higher ability to penetrate the EPS when compared to Listerine® Antiseptic. Furthermore, it is important to notice that in the present study by exposing for 24 continuous-hours the toothbrush heads to saliva we created a very robust biofilm. Therefore the bacterial load achieved may not be fully representative on what can be found on the toothbrush normally used by the typical toothbrush use.

Lastly, both the antimicrobials and the concentrations used in this study are categorized by the FDA as generally recognized as safe (GRAS) and they have not been reported responsible for untoward human health effects. Considering that bacterial contamination of regularly used toothbrushes

can last up to 48 h,³⁰ we can also speculate that the use of antimicrobials agents³¹ prior to the daily brushing activity could reduce bacteria that may have colonized during the hours of non-use³² as well as eliminate bacteria coming from the oral cavity after brushing activity.

5. Conclusion

Although the results of our study have shown that 3% hydrogen peroxide has greater efficacy in reducing bacteria deposited on used toothbrush, different antimicrobial agents and/or devices can be recommended for decreasing the bacterial load present on toothbrushes. This information adds to the knowledge and recommendation that patients should be strongly encouraged to disinfect toothbrushes by means of using antimicrobial solutions easily available at home.

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