Efficacy of ozonated water to remove bacteria from surfaces: A comparative study with commercially available soap solutions

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Introduction

Cleanliness of hand surfaces is of primary importance to keep good hygienic practices and prevent infection. Hand surface cleanliness could prevent several infections as the disease causing microorganisms can get spread via fomites and other contaminated surfaces such as hand washing sinks. Some highly publicized infection cases in Canadian hospitals lead to the formation of new National Standard of health care facilities called Z8000 released in 2011.This standard primarily aim to limit patient, staff and visitor exposure to potential infection. Between December 2004 and March 2006, bacterial infection outbreak caused several deaths. Although the causes of the infections were different, there was one common factor in all of these cases: the sinks. Considering this situation and as a positive step to reduce these kinds of infections in health care facilities, Franke Kindred has developed a new sink that uses ozonated water to reduce bacterial population and therefore contamination via handwashing sinks. However, Franke, Midland, ON does not have the expertise and facilities to professionally test the efficacy of the sink to reduce bacterial growth and therefore approached Lakehead University for help and advice. Lakehead has agreed to conduct a study on this. This project will extensively study the efficacy of ozonated water from the newly developed hand washing sink to remove microbes from hand surfaces in comparison to commercially available hand-washing soap solutions. This study will monitor bacteria on hand surfaces before and after the washing with water from this sink and with several commercially available soap solutions of different dilutions.

Objectives

Distilled and autoclaved water will act as the control for all these experiments. The objectives of the project are: 1) Data on the efficacy of ozonated water as a hand washing solution to reduce/remove microbes from hand surfaces, 2) Comparative data on the efficacy of ozonated water to the commercially available hand washing soap solutions to remove bacteria from hand surfaces, and 3) Photographs of bacterial colonies on growth media such as petridishes. The results will help the company to validate the efficacy of the product.

Methods

Five different hand washing treatments (ozonated water, deionized water, soap and tap water, ozonated water and soap, and tap water) and one drain treatment were utilized to study the reduction in total viable bacteria counts. Standard microbiological techniques were employed during the experiment such as sterilizing equipment and media in an autoclave for 30 minutes at 121ºC, changing gloves frequently and working in a biosafety cabinet or near an open flame. Sterile swabs were taken from hands before and after each treatment and diluted in sterile deionized water. The pour plate method was followed and replicate samples were plated for each dilution. Nutrient agar media was used to grow the heterotrophic bacteria and plates were incubated at 25ºC (±2ºC) for 3-6 days. The number of viable bacterial or colony forming units (cfu) were counted for each plate and plates ranging in 30-300cfu were used to calculate the total reduction in viable bacteria.

Results

Mean total viable bacteria counts for before and after treatments are displayed in Table 1. Total viable counts ranged from 45 – 4.20×10^4cfu/ml for before treatments and 17.5 – 8.28×10^3cfu/ml for after treatments. This same information is represented in Figure 1 with $\log_{10}$ transformed bacterial counts and standard deviation. The standard deviation for the drain treatment could not be calculated because there was only one successful trial. Pictures of the plates from some of the trials are shown in Appendix A.

Table 1. Mean total viable bacteria counts (cfu/ml) for before and after treatments

Treatment	Before	After
Ozonated	16950.0	2412.5
Deionized	110.0	80.0
Soap	45.0	17.5
Ozonated + soap	42040.0	8275.0
Tap	945.0	1322.0
Drain	2470.0	250

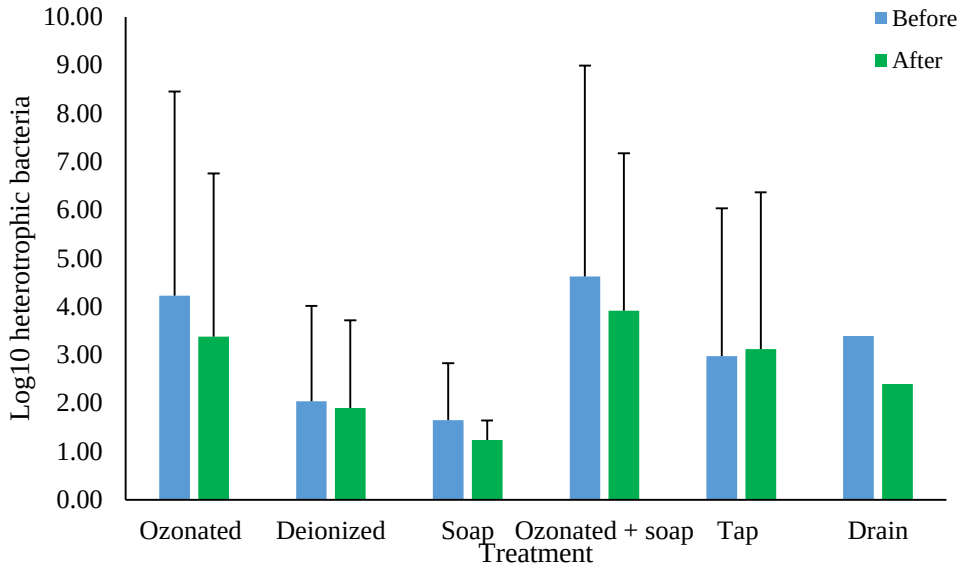


Figure 1. Log_{10} transformed bacterial counts before and after with standard deviation

Figure 2 shows the mean percent reduction in total viable bacteria with standard deviation (excluding drain treatment). The drain treatment had the highest reduction in bacteria although this is just based off of one trial. Out of the hand washing treatments the ozonated water had the highest percent reduction with an average of 80.4% and deionized had the lowest reduction at 14.7%. The tap water treatment which acted as the control exhibited the most variability in reductions.

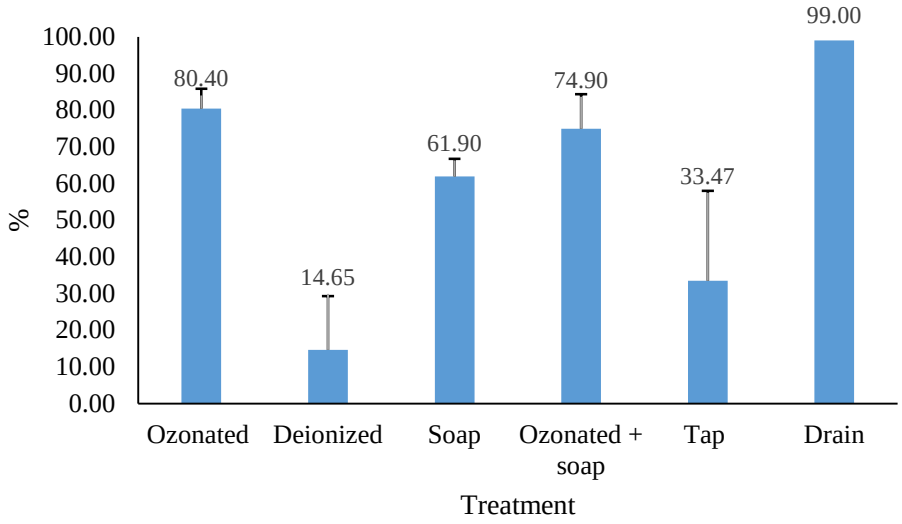


Figure 2. Mean total viable bacteria reduction (%) with standard deviation

Statistical Analysis

To determine if there was significant difference in mean bacteria reduction between all treatments a goodness-of-fit test was run using a chi square distribution. The null hypothesis being that there is no effect of hand washing treatments on total viable bacteria reduction and the alternative that there is an effect. The assumption that no cell expected frequency should be less than 5 when degrees freedom is 1 was met. The results of this analysis are shown below in Table 2.

Table 2. Summary of goodness-of-fit tests for hand washing treatment effect on total viable bacteria reduction. A significance level of 0.05 was used.

Treatments	χ^2 statistics	p
Tap vs Ozonated	19.342	<0.001
Tap vs Deionized	7.361	<0.01
Tap vs Soap	8.475	<0.001
Tap vs Ozonated+soap	15.839	0.001
Ozonated vs Deionized	45.482	<0.001
Ozonated vs Soap	2.405	0.12
Ozonated vs Ozonated+soap	0.195	0.66
Deionized vs Soap	29.165	<0.001
Deionized vs Ozonated+soap	40.537	<0.001
Soap vs Ozonated+soap	1.235	0.27

Conclusion

As part of the first objective we collected data on the efficacy of ozonated water as a hand washing solution to reduce/remove microbes from hand surfaces. Average viable bacteria counts decreased from $1.70x10^4$cfu/ml to $2.41x10^3$cfu/ml an overall reduction of 80.4%. This reduction was found to significantly different from the tap water control (χ^2 =19.342, p<0.001). Furthermore, the reduction in drain bacterial counts was 99% ($2.47x10^3$cfu/ml to $2.50x10^2$cfu/ml). This difference could not be tested statistically due to lack of successful trials. It is apparent that ozonated water is an effective solution to reducing and removing microbes from hand surfaces.

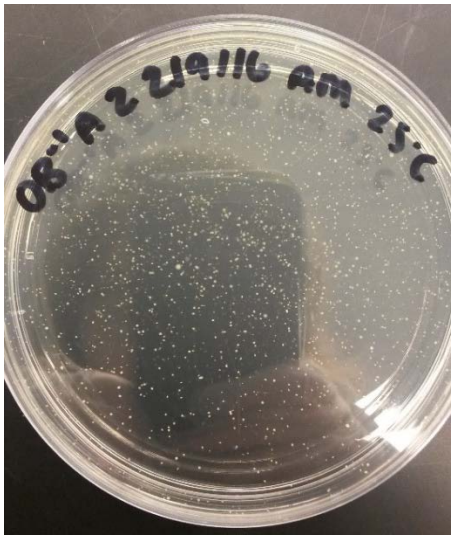
Data was collected for the second objective of the efficacy of ozonated water to commercially available hand soap solutions to remove bacteria from hand surfaces. The soap solution tested was Nature Clean (unscented). The reduction in bacteria for the soap treatment was on average 61.9% compared to 80.4% for ozonated water. This difference was not found to be significant (χ^2 = 2.405, p = 0.12). Washing hand surfaces with both soap followed by ozonated water did not cause a further significant reduction in bacteria (74.9%) compared to washing with ozonated water alone (χ^2 =0.195, p = 0.66). Although the reduction in bacteria from hand surfaces between ozonated water and the soap solution was not found to be significant, ozonated water could potentially be a more environmental friendly solution since it does not contribute harmful

chemicals such as triclosan, sodium lauryl sulphate, parabens, or 1,4-Dioxane into wastewater. This would lower costs of wastewater treatment and decrease the impacts on aquatic ecosystems. Therefore, it is highly recommended that Ozonated water from the newly constructed ozonizer may be used in hand washing sink in all the commercial and other facilities.

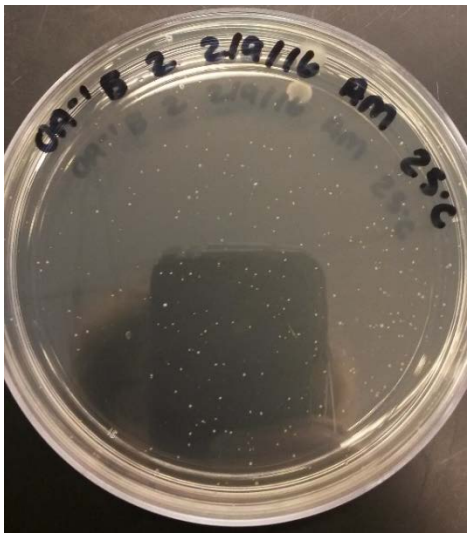
Appendix A

Total viable bacteria plate pictures taken 2/12/2016

Ozonated water before:



Ozonated water after:

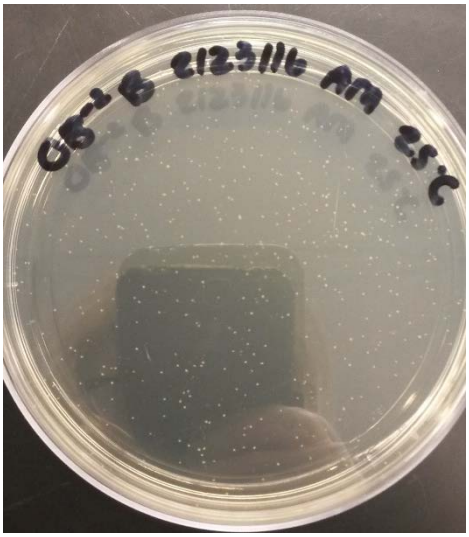


Tap water before:

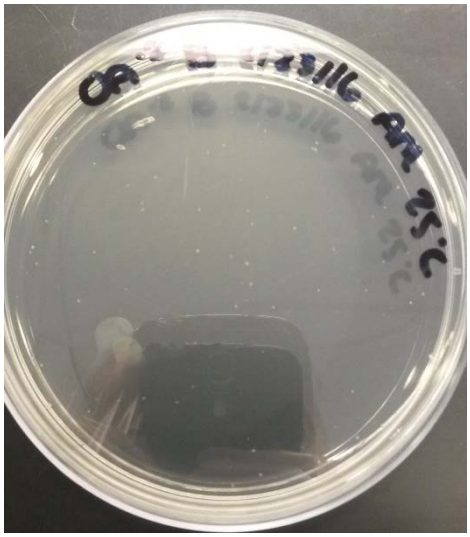
Tap water after:

Total viable bacteria plate pictures taken 2/26/2016

Ozonated + soap before:

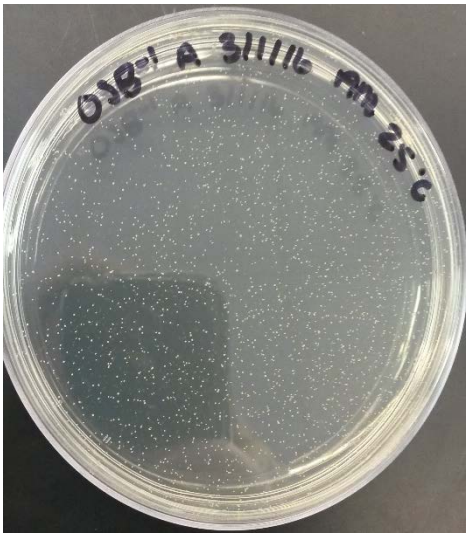


Ozonated + soap after:



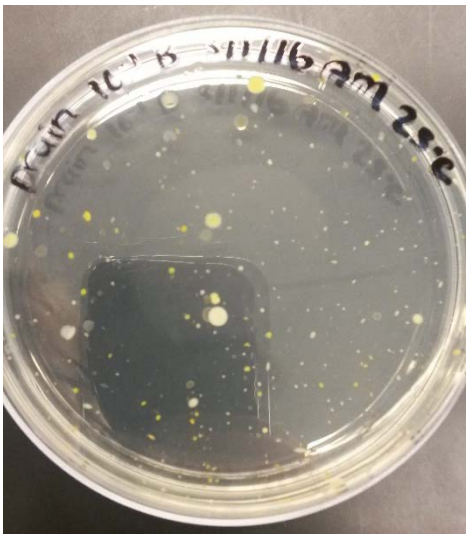
Total viable bacteria plate pictures taken 3/4/2016

Ozonated + soap before:



Ozonated + soap after:

Drain before:



Drain after: