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Bacterial Load Consistency Among Three Independent Water Distribution Systems

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Summary

Clean drinking water is an essential component to maintaining public health. Methods used to purify drinking water include the addition of disinfectants, chemical methods of control, such as ozone or ultraviolet radiation, and physical methods of control, such as filtration. Disinfectants and filtration are the most widely used methods to ensure high-quality drinking water. However these methods do not sterilize the water, and there are still concerns as to whether or not some methods are better than others at reducing bacterial loads. This study tested water from traditional drinking fountains, Poland Springs® water cooler dispensers, and from Halsey-Taylor Filtered Single HAC Coolers with Hydroboost® Bottle Fillers. Water samples were collected from each source types on various days, plated onto Nutrient agar, and analyzed to count CFUs/mL for each source. We found that there is no statistical difference in the mean bacterial CFUs/mL among the three water sources tested. We conclude that among samples tested, no one water source is superior to the others in terms of bacterial load. The continued application of studies like this one to available water sources may inform consumers on whether certain water sources have healthier bacterial compositions than others. Should differences exist, these studies could inform consumers on how to better attain high-quality drinking water.

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Introduction

Access to clean drinking water is one of the most important public health initiatives. Microbial processes, such as biofilm formation, often contribute to the deterioration of water quality, particularly among water distribution systems (1). Betterment of the water supply coming from a water distribution system aims to reduce microbial presence and ensure high-quality drinking water. Achieving a high-quality drinking water supply with low numbers of microorganisms can be accomplished using a variety of methods, including filtration and the addition of disinfectants like chlorine (2). In the city of Lowell, MA, the drinking water supply comes from four groundwater wells, which draw water from the Grand River watershed of the Merrimack River. The city's drinking water supply follows the EPA's safe drinking water regulations. The water is tested annually (at a minimum), ensuring safe-for-consumption contaminants, including microbes, disinfectants and their by-products, and inorganic matter (3).

The main methods that the City of Lowell, MA uses to disinfect the drinking water supply are filtration and chlorination (3). In a city or town water treatment processes, filtration aims to coagulate large particles for removal and eliminate potentially pathogenic microorganisms from the water (4). Chlorination is employed because it is effective at reducing the numbers of microbial contamination in water, including for organisms such as hemorrhagic *E. coli* (5). However, other methods also exist to reduce or remove microorganisms, pathogenic or otherwise, from the water supply. For example, Poland Spring® drinking water undergoes an extensive disinfection, using line sanitation processes to remove microbial contaminants. Poland Spring® also uses microfiltration, which utilizes filters that remove particles 0.2 microns or larger in diameter, as well as ozone and ultraviolet disinfection (6).

In recent years, traditional drinking fountains have also begun to reduce microbial contaminants with further supplementation. One such water distribution system that employs additional antimicrobial techniques for removing microorganisms in the water is the Halsey-Taylor Filtered Single HAC Cooler with Hydroboost® Bottle Filler. This water distribution system contains proponents which are integrated with silver ions. These silver ions are used to reduce the presence of mold and mildew (7). In an iodized form, silver particles are capable of causing protein and DNA damage, as well as cell membrane detachment from the cell wall,

and irreparable structural damage to Gram-negative organisms, such as *E. coli* (11).

While current methods used to remove bacteria from drinking water sources are effective, it remains unclear how much bacteria remain present within the water after treatment. To explore this question further, samples of water were taken from four traditional drinking fountains dispensing Lowell city water, two Poland Springs® water cooler dispensers, and two Halsey-Taylor Filtered Single HAC Coolers with Hydroboost® Bottle Fillers. We hypothesized that the water taken from the Poland Springs water cooler dispenser and from the Halsey-Taylor Filtered Single HAC Cooler with Hydroboost® Bottle Filler would show statistically less bacteria per milliliter of water sample tested than the traditional drinking fountains. Data analyses of the samples revealed no difference in bacterial load across all samples tested. Microbial growth occurred from all of the water samples taken from each source type, and there was no statistically significant difference between the bacterial loads among the three sources tested. These findings show that bacteria are present in drinking water even after purification has occurred but that one purification method does not appear to be remarkable over the others.

Results

The samples tested were obtained from four traditional drinking fountains, two Halsey-Taylor Filtered Single HAC Cooler with Hydroboost® Bottle Filler stations, each with a mouth piece and bottle filler for obtaining water, and two Poland Springs® water cooler dispensers. The mouth pieces were not disinfected prior to sampling as it is unlikely that a student would also do so before drinking. For randomization of collection time, some water samples were collected at 6:30 a.m. and11:00 a.m.

In terms of counting colonies, plates containing between 25-250 colony forming units per milliliter (CFUs/mL) of water are considered countable. In total, two of the traditional drinking fountain water samples grew greater than 250 bacterial colonies on the Nutrient agar. These samples were serially diluted 1:10 in 0.85% Isotonic Buffered Blood Bank Saline. This was done in order to ensure that the bacterial growth on the Nutrient agar plate was easily measurable. Isotonic Buffered Blood Bank Saline was used because it was readily available, sterile, and the saline concentration was not assumed to be high enough to adversely affect the growth the bacteria present in the water samples. This also occurred for one of the Poland Springs® water cooler samples. The samples that underwent a 1:10 serial dilution can be seen in **Table 1**, designated by an asterisk (*).

To determine bacterial load on the water, one milliliter (mL) of each water sample from each source was pipetted onto its own nutrient agar plate and the plates were incubated at 35°C for 24 hours. After 24 hours, the Nutrient agar plates were observed for visible growth of bacterial colonies, and bacterial CFUs/mL were calculated. The colony count data was organized using Microsoft Excel (8). Samples labeled “A” were from the Halsey-Taylor Filtered Single HAC Cooler with Hydroboost® Bottle Filler stations, samples labeled “B” were from the traditional drinking fountains, and samples labeled “C” were from the Poland Springs® water cooler dispensers. All control plates containing no water sample had no growth of bacteria throughout testing.

Pseudoreplicates are present in this experiment, due to samples being collected from the same source at the same time. This makes the number of true replicates from each source are 4, 4, and 2, respectively. Since

Halsey-Taylor Mean CFUs/mL	Traditional Drinking Fountain Mean CFUs/mL	Poland Springs Mean CFUs/mL	Control Mean CFUs/mL
(A1) 0	(B1) 395*	(C1) 0.67	0
(A2) 3.88	(B2) 567.91*	(C2) 35.44*	
(A3) 6	(B3) 4.25		
(A4) 2.44	(B4) 0.25		

Table 1. Pooled measurements for each of three sources. The data represent the mean bacterial load/mL of water taken from each source. (*) Indicates that these samples underwent a 1:10 serial dilution in 0.85% Isotonic Buffered Blood Bank Saline in order to obtain a Nutrient agar plate that was considered easily countable, i.e. contained between 25-250 CFUs/mL. A, B, and C indicate the letter designations used to blind the samples when being tested.

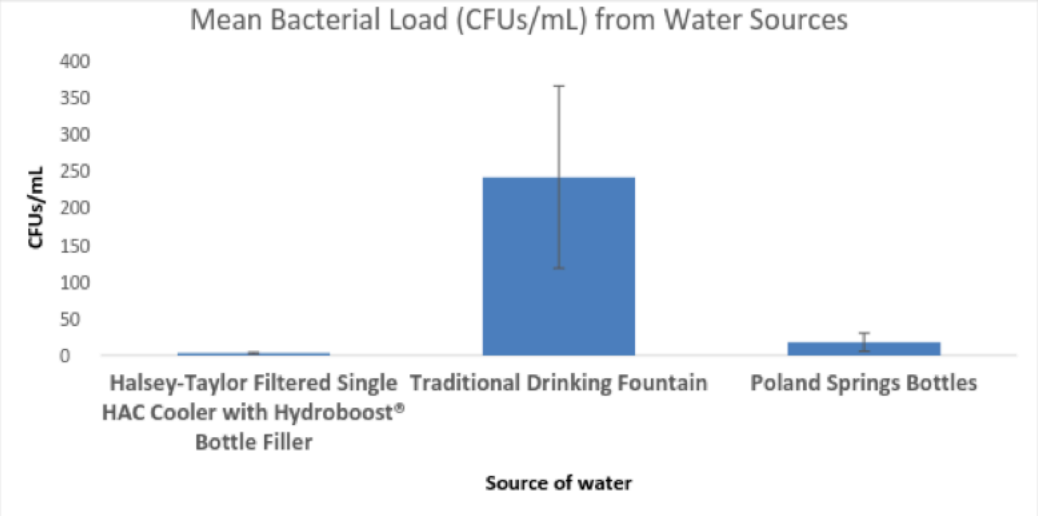

Figure 1. Bar plot of mean bacterial load. The standard error is shown. The greatest bacterial load can be seen in the Traditional Drinking fountain, followed by the Poland Springs Water Cooler Dispenser, and then the Halsey-Taylor Filtered Single HAC Cooler with Hydroboost Bottle Filler.

there are pseudoreplicates, the data were pooled from each source, giving the mean bacterial load per mL of water from each source sample (**Table 1**).

We discovered that the traditional drinking fountain has the largest mean CFUs/mL of bacteria present, followed by the Poland Springs® water cooler dispenser and then the Halsey-Taylor Filtered Single HAC Cooler with Hydroboost® Bottle Filler stations (**Figure 1**). With three water sources being tested, an ANOVA would have been the best statistical test to use. However, there were large variances between the groups, supported by a Levene's test (p-value is 0.00035). Since the assumptions of the ANOVA were violated, it was determined that a Welch's ANOVA could be used to determine statistical significance based on the large difference in variance between the groups, as well as a Nested ANOVA, which can account for pseudoreplicates in the data. Statistical significance was set at $p < 0.05$, meaning that if a p-value produced was less than 0.05, the data would support rejection of the null hypothesis, indicating that there is in fact a difference in mean bacterial load (CFUs/mL) between the three water sources tested. Using R-stats, the Welch's ANOVA produced a p-value of $p = 0.43$, and the Nested ANOVA produced a p-value of $p = 0.132$ (9), respectively. In both cases, $p > 0.05$, indicating that these tests fail to reject the null hypothesis, which states that there is not a difference in bacterial load between the three water distribution systems.

Discussion

The p-value for both the Welch's ANOVA and Nested ANOVA produced $p > 0.05$, indicating that there is no statistically significant difference among the mean bacterial CFUs/mL from the three tested water sources. These findings demonstrate that bacteria are still present in drinking water after purification processes; however, it cannot be said that there is a difference in mean bacterial load among the three water distribution systems. This information may prove useful to consumers that are concerned about the quality of their drinking water. The purification methods used by the City of Lowell, MA, which consist of chlorination and filtration, Poland Springs® Company, which adds microfiltration to its purification process, and the silver-ion coated components of the Halsey-Taylor Filtered Single HAC Cooler with Hydroboost® Bottle, all appear to result in similar amounts of bacteria present in purified water.

While data analyses fail reject the null hypothesis, there are several limitations inherent in this study. The first limitation arises due the small sample size and the presence of pseudoreplicates in the experiment. When looking at **Figure 1**, it is difficult to say that there is no statistical difference among the mean bacterial load between the three systems. With the Levene's test supporting that there is a difference among the variance between the samples, it can be said that the sources are different. However, this difference is in terms of variance and not mean bacterial load. This warrants further investigation involving a larger sample size and a lower number of pseudoreplicates. Additionally, it would be worth exploring whether or not there are differences among the mean bacterial load between the systems with respect to the time of day of collection since it known that development of biofilms occurs overnight when water is stagnant, which allows for bacterial conglomerates to form. As water flow resumes during the day through usage of the drinking fountains, these biofilms break apart due to the force of water moving through the water

delivery lines.

Another limitation of this study comes from the media used to grow the bacteria. While Nutrient agar is considered to be a complex media, meaning it is capable of growing a variety of bacterial species, it is not particularly useful for growing bacteria that are fastidious and require a more enriched medium for growth. Traditional drinking fountains often accumulate a buildup of respiratory microbes, many of which fall into this fastidious grouping. Therefore, a more enriched media, such as Chocolate agar, should be used for future testing. This would help ensure that all of the potential bacteria present in the sample are accounted for. It may also be beneficial to incubate some of the samples in low oxygen conditions to allow for growth of any anaerobic bacteria that may be present. This limitation was demonstrated when a Gram-stain, which is a differential staining mechanism that allows for determination of bacterial cell size, shape, and cell wall composition, was performed on one bacterial colony from source C1. The Gram-stain result showed a pleomorphic Gram-positive bacillus. Further identification may have been possible if the specimen were incubated anaerobically, as this is a typical Gram-stain result for bacteria belonging to the *Propionibacterium* genera, which are anaerobic (10). Moreover, other types of selective and differential media could also be used to aid in further identification of the types of bacteria present in the sample. Identification of the bacteria would be useful in knowing whether or not the organisms present in the water are pathogens.

A final limitation of this study is that there is no overall comparison of how effective these purification methods are in relation to the amount of bacteria present in the drinking water pre-treatment, *i.e.* straight from the source. It would be worth exploring what the overall reduction of microbial load is from the original water sources due to the purification methods employed; however, it was not possible to obtain a sample from upstream of the city of Lowell's water utility shed, nor from the Poland Spring's® water source, it can be assumed that the microbial numbers in the water post-filtration are similar for both, as all drinking water must adhere to strict EPA water quality standards.

Materials and Methods

Three water samples were collected from each water source (Halsey-Taylor Filtered Single HAC Cooler with Hydroboost® Bottle Filler, traditional drinking fountain, and Poland Springs® water dispenser) in sterile, capped test tubes (Pyrex tubes 13X100MM 529703). The sources designated A, B, or C to indicate source (source A is the Halsey-Taylor Filtered Single HAC Cooler with Hydroboost® Bottle Filler, source B is the traditional drinking fountain, and source C is the Poland Springs® water dispensers). Water samples were capped and then inverted in order to equally distribute the sample in the tube. One milliliter of the each water sample was pipetted onto its own Nutrient agar plate (Frey Scientific lot 7116). The plates were covered and left to sit for 5 minutes to allow for diffusion of sample across the plate. Control plates were also set up for each round of testing. The control plates were Nutrient agar plates that had been opened for the duration of time it took to pipet a water sample onto the plate. All plates were then incubated at 35°C for 24 hours. Capped water samples were stored in the laboratory freezer for twenty-four hours for additional testing, if needed.

The following day, the plates were observed for growth and colony-forming units per milliliter (CFUs/mL) of water were calculated. For samples that had over 250 CFUs/mL on the Nutrient agar plates, a 1:10 serial dilution was performed from the original capped water sample. One-half milliliter of the capped water sample was pipetted into 4.5 mL of sterile, 0.85% Isotonic Buffered Blood Bank Saline (Thermo Scientific Lot 673910). This was done in order to ensure that the bacterial growth on the Nutrient agar plate was easily countable. 0.85% Isotonic Buffered Blood Bank Saline was chosen as the dilution medium because it was readily available, sterile, and the saline concentration was presumed to not be high enough to adversely affect the growth the bacteria. When serial dilutions were performed, one milliliter of the serially diluted sample was pipetted onto a new Nutrient agar plate. The plate was allowed to sit for 5 minutes to allow diffusion of the sample, and was then incubated at 35°C for 24 hours. Colony counts were performed for serial dilution plates after twenty-four hours of incubation. The CFU number was multiplied by the dilution factor to approximate CFUs/mL of the original sample. This procedure was repeated for three rounds of testing on three different days.

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