Bacterial Growth in Personal Stainless Steel Water Bottles: How Often Should You Clean Your Bottle?

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Abstract

Background: There is a general understanding and knowledge among reusable personal water bottle users that there are hazards, such as bacterial growth, associated with poor water bottle hygiene practices. Currently, there is no information associated with outbreaks or cases of illness stemming from poor hygiene on personal water bottles. This may be due to lack of awareness that users have become ill from their own water bottle and have failed to report it. Results from previous studies on personal water bottles have indicated that there is a relationship between higher microbiological counts and the interval between cleaning times; the longer water bottles are left unclean, the higher the microbial count.

Methods: 29 randomly sampled stainless steel personal water bottles were swabbed at the mouth piece and 1 brand new personal stainless steel bottle was used as a control. Personal water bottle users were provided with an in-person electronic survey at the time of sample collection. The swabs were plated following the 3M Aerobic Plate Count method and incubated for a total of 72 hours. Plates were counted after 24 hours and 72 hours.

Results: There was no statistically significant difference between the aerobic bacterial levels (CFU) of personal stainless steel water bottles that were cleaned within one day and those cleaned within a month but more than one day based on the Independent Sample T-test. There was also no statistically significance difference between the aerobic bacterial levels (CFU) of bottles that were rinsed with tap water and those cleaned with soap and water based on the Independent Sample T-test.

Conclusion: Based on the results, stainless steel water bottles are not required to be cleaned frequently. It also appears that there is no difference between cleaning with soap and water and just rinsing the bottles with tap water. Despite results showing no statistical difference to support more frequent cleaning and more thorough cleaning practices, these behaviours should be encouraged to prevent and minimize the risk of potential exposure to harmful pathogens.

Keywords: *personal water bottle, aerobic bacteria, hygiene, cleaning, aerobic plate count, sanitation*

INTRODUCTION

Water consumption is a necessary part of life. As a result of smart marketing practices and constant promotion by corporations, bottled water has now become the preferred way of drinking water on the go. Although this has led to the increased use of disposable bottles, there have been campaigns to decrease the environmental impact of these bottles by using reusable and durable metal water bottles (1). As a result, the use of personal water bottles in sports, school, work, or travel is highly prevalent (2). Although using reusable water bottles are better for the environment, the health of the users is in question as it is likely that many do not consistently practice proper hygiene.

Since the health and safety of the public is of paramount concern for environmental health officers and other public health professionals, information provided to the public on the hazards of poor personal water bottle hygiene is essential. As such, the public should be informed that personal reusable water bottles can provide a conduit for users to become ill due through factors such as: poor or rare cleaning between uses, exposure to room temperature for long periods, exposure to air particles, particulates, and possibly pathogens when opened, and dirty hands or water sources where they are filled.

There has been increased awareness to drinking water quality coupled with heavy regulation over the years focusing on water source, distribution, and bottled water manufacturing (1). At this time, there are no hard guidelines recommended for personal water bottle cleaning frequency and sanitation.

LITERATURE REVIEW

Bacterial Growth and the Water Bottle Environment

A reusable water bottle provides the perfect environment for bacterial growth. Water bottles are able to trap in moisture and humidity, and are subject to contamination of bacteria due to frequent contact with the users' hands and mouth. Additionally, water is usually stored at temperature ranges between 4-42˚C for long periods. These all correlate with known food safety problems and are thus in the top three reasons for spreading disease (3).

Biofilm formation occurs when there is an overgrowth of bacteria, forming thin layers. Since bacteria can adhere to any part of a water bottle, it is expected that bottles with poor hygiene will have a greater prevalence of biofilm formation (4). Sanitization of bottles with biofilms will be ineffective in eliminating pathogens as the film provides a protective coating for the bacteria (5). As described in the *Food Retail and Food Services Code*, it is necessary to properly wash bottles and surfaces with detergent to fully eliminate biofilm, allowing access for sanitizer to eliminate the remaining resilient pathogens (5).

A study conducted by former BCIT student, Sophia Fantillo, assessed drinking water quality in water refill stations across BCIT. Her results indicated that there were increased levels of heterotrophic bacteria from a Heterotrophic Plate Count (HPC) analysis (6). Although this study did not focus on water bottles, it highlights the fact that water refill stations have the potential to provide a pathway in which the mouthpiece can come in contact with, and become contaminated by, a water source.

Water Bottle Hygiene Practices and User Behaviour

There is a general understanding and knowledge among reusable water bottle users that there are hazards, such as bacterial growth, associated with poor water bottle hygiene practices. However, there is a lack of information readily available to truly demonstrate the severity which poor water bottle hygiene poses, and this deficit of information has contributed to attitudes of '*I have not become sick yet so what I am doing is of no consequence*' leading to a false sense of security. As many are aware, there are pathogens associated with the handling of water bottles and the drinking water within them. Despite the general awareness of biological hazards associated with water bottles, there is a larger focus on chemical leaching from commercially bottled water and reusable aluminum bottles in particular (7).

Water bottle manufacturers such as Rubbermaid®, Contigo®, and Thermos® have independently indicated instructions in their manufacturer's information packet for care and use of their water bottle (8–10). The information sheets state that bottles should be washed with warm soapy water and air dried (8–10).Although these do come with the product, users often do not read them or follow the labels' instructions. Additionally, these instructions rarely indicate a sanitizing step, minimum cleaning contact times, and types of detergent to use.

A study project using surveys conducted by a group of students from the University of Michigan (UoM), analyzed water bottle user behavior in a small sample of students (11). At the conclusion of their study, they found that there was a lack of education regarding the quality and safety of source water, and that students did not clean their bottles in between uses as it was an inconvenience (11). Several recommendations made as a result of their study may have resulted in increased use of reusable water bottles and proper hygiene. These included recommending the school provide free reusable water bottles, installing cleaning stations around school facilities, and educating students on water quality and hygiene practices to ensure their water bottle is safe to drink from. These suggestions can also be used to instill good habits in reusable water bottle users.

Bottled Water and Outbreaks

Unsanitary practices are the root cause of outbreaks in commercially bottled water. According the Center of Disease Control and Prevention (CDC); bottled water outbreaks are not commonly reported but have occurred (12). One of the more recent outbreaks in 2010 was an acute gastrointestinal illness due the bottle becoming contaminated at point of use from an unknown chemical agent (12). Much of the outbreak data collected by special agencies such as CDC, Public Health Agency Canada (PHAC), and Health Canada (HC) focuses on drinking water source and distribution systems. There is currently no information associated with outbreaks or cases of illness stemming from poor hygiene on personal water bottles. This may be due to lack of awareness that users have become ill from their own water bottle and have failed to report it.

Drinking Water and Bottled Water Guidelines and Regulations

Since bottled water sold in a store is considered a food product, regulations and guidelines are in place, governed by HC, Canadian Food Inspection Agency (CFIA), and the Canadian Bottled Water Association (CBWA) (13).

Drinking water is heavily regulated by the *Drinking Water Protection Act and Regulations*. This piece of legislation covers requirements for drinking water operators and suppliers which they need to maintain to ensure that their water is safe for consumers to drink and use (14). Under *Food and Drug Regulations*, in Division 12, Part B, bottled water is considered a food product and regulated through microbiological standards, appropriate treatment procedures, and labelling specifications (13). Through these legislations, drinking water has been safe for consumers to drink and use. Although these legislations have decreased the spread of disease and prevalence of outbreaks, these acts and regulations are limited as they only focus on the drinking water itself and commercially bottled water products. These legislations fail to encompass the water in personal reusable water bottles. This may be attributed to the fact that it will be difficult and impractical to enforce these types of legislations on users.

Although there has been no legislation found for personal water bottle hygiene, the microbiological levels of aerobic bacteria grown for this study were compared to the "Aerobic Colony Count Recommendations for Environmental Surfaces" in the *Food Quality Check Program Microbiological Recommendations* developed by the BC Public Health Microbiology & Reference Laboratory (15). This program is used by Environmental Health Officers in the field to educate food service establishment operators. The recommended limits outlined in this program are relevant to this study as the parameters are used to ensure that environmental surfaces are adequately cleaned and sanitized to prevent the potential for bacterial growth.

Microbiological Testing of Water Bottles

To determine the safety of drinking water from a water source, the following tests are commonly used: total coliform counts (TCC), fecal coliform counts (FCC), and *Escherichia coli* coliform counts (ECC) (16,17). TCC tests for the presence of all coliforms, fecal and naturally occurring, and it indicates whether or not the water treated has been disinfected properly (18). FCC and ECC indicates if there has been a recent contamination of the water source from fecal matter which may contain other bacteria, viruses, and disease-causing bacteria (18). These tests on drinking water focus mainly on fecal contamination as that is easily and commonly spread through water, causing gastrointestinal diseases. Since there have been numerous studies conducted on the quality of water itself, there has been a lack information regarding the bacterial levels at the point of contact for the user which is the mouthpiece.

A non-peer reviewed study, funded by a treadmill review website, tested various types of reusable water bottles (19). The results of this study indicated that there is a high number of bacteria located at the mouthpiece. They demonstrated that bottles with smaller openings were difficult to clean and contained a higher number of bacteria in comparison to the wider mouth bottles (19). Since this study contained a small sample size of 12 and was not peer-reviewed, the results of this study cannot be viewed or used with confidence, highlighting the lack of supported evidence for bacterial levels on the mouthpiece of reusable water bottles.

Aerobic Plate Count

APC, or Aerobic Colony Count (ACC), measures the presence of aerobic bacteria on surfaces (20). This test commonly indicates the sanitation levels of food contact surfaces. Limitations of ACC include that it will not identify specific pathogens that may cause diseases and provides a low level of detection (20). An advantage to this method is that samples are easily collected via the swabbing technique and do not require a large sample (20).

Water Bottle Studies

In a study conducted by former BCIT student, Vanessa Ouellette, the drinking water quality in varying plastic personal water bottles was tested through water sample collection and heterotrophic plate counts (HPC) (2). HPC is a method commonly used to measure the number of heterotrophic bacteria that are present in a water sample collected from various types of water sources (21). In her study, she focused mainly on the water quality contained in either soft or hard reusable plastic water bottles. To supplement, she conducted a survey to assess the water bottle hygiene behaviors of users. Her results indicated that there was a small correlation between higher microbiological counts and the interval between cleaning times; the longer water bottles are left unclean, the higher the microbial count (2).

Aside from Ouellette's study, there appears to have been only one other study that focused on the water quality in water bottles using a bacterial analysis. This study, conducted by Ryan *et al.*, focused on an elementary school in Alberta where students were encouraged to use a water bottle in school, but were not encouraged to clean them at home (22). It was discovered that some students would use their bottles without cleaning them for months, and an analysis of these sample showed that the maximum of 500 CFU/mL outlined in the Guidelines for Canadian Drinking Water Quality was exceed in approximately 64.4% of the water samples collected (22).

Both of these studies indicate that a lack of water bottle hygiene has contributed to the exponential growth of bacteria in the water contained within the water bottles.

Stainless Steel Versus Plastics

Reusable water bottles are commonly made from food-grade material. Common materials include soft or hard plastics, metals such as aluminum and stainless steel, and glass. In the food service industry, stainless steel is an ideal material to use for food preparation as this contact surface is easy to clean, durable, and does not crack like other materials such as plastic and wood (5). Additionally, stainless steel items are known to have antimicrobial properties and do not leach into the water, unlike plastic and aluminum items (23). Although stainless steel has many great characteristics, bottles may corrode over time due to excessive use of bleach as a sanitizing agent (24).

Plastics are more porous than stainless steel and more prone to crack formation. Cracks in material provides a shelter for bacteria to grow in as these spots become difficult to reach when cleaning (25). Although plastics are more cost-effective for the user, it has been recommended to purchase metal water bottles – particularly stainless steel due to the reasons mentioned above.

For this study, aerobic bacterial growth on stainless steel water bottles was assessed to determine sanitation levels; Ouellette's study has already focused on soft and hard plastics, and there is little information determining bacterial growth between stainless steel materials and water (2).

Research Project Purpose and Goals

The purpose of this research project was to determine how often personal stainless steel water bottles need to be cleaned and which methods were most effective in order to reduce levels of bacteria to the levels outlined in the recommended Aerobic Colony Count Recommendations (15).

The goal of this research project was to provide relevant information to the public, as the results will provide guidance as to the importance of proper hygiene of reusable water bottles to ensure safe drinking water.

METHODS

This research project was conducted through both a microbiological test and a survey. The standard methods for measuring aerobic bacteria were based on the procedures outlined by 3M (26,27). The following materials were used:

Item	Quantity
3M Quick Swab	30
Cooler with Ice Packs	1
3M Petrifilm for Aerobic Count Plate	30
Counter	1
Google Forms survey by Google	1
JMP 13 – Statistical Software	1
Microsoft Excel by Microsoft Office	1

Survey

A survey, generated on Google Forms, was conducted electronically and delivered in person via tablet at the time of sample collection. A script was used to ensure that information provided to each participant was consistent. A short cover letter was also provided to allow the participants to gain some knowledge of the study and confidentiality information. Each participant was also given an opportunity to receive results of the study. Each participant's survey and collected sample swab were numbered or lettered accordingly.

Sample Collection

Samples were collected using aseptic techniques to reduce the chances of contamination. Prior to collection, the researcher washed her hands and labelled the 3M Quick Swab sample accordingly. Swab collection followed the dry swabbing method (27). A surface area of 100 cm^2 was swabbed around the mouthpiece.

Aerobic Plate Count (APC)

The swabbing and plating method followed the procedures outlined by 3M for swab collection and plating (27,26). The purpose of this method is to enumerate aerobic bacteria swabbed from water bottles. After dry swabbing the mouthpieces, the samples were plated onto a 3M Petrifilm for Aerobic Plate Count. After samples were plated, they were incubated and counted at 24 and 72 hours at room temperature. Colonies that grew were stained red. Plates were then compared to plate figures in the Interpretation Guide to determine if they were countable or were too numerous to count (28).The equipment for this method required no calibration. The controls for this study included swabbing a stainless steel water bottle that was brand new and had not been washed or used.

Inclusion and Exclusion Criteria

Participants that used stainless steel water bottles were selected randomly. To ensure that users were randomly selected, the researcher approached a wide range of participants at various locations at the Burnaby BCIT Campus (SW1, SE2, SW3, SE12, SE14, and SE6) with a poster and sample collection materials. The poster stated, "Want to know what is growing in your water bottle?". Users with bottles that were made up of plastic, glass, or aluminum and stainless steel water bottles that had non-stainless steel mouthpieces were excluded from this study. In addition, ENVH 8410 students were also excluded from this study as they were aware of the anticipated results.

Ethical Considerations

The survey portion of the study was performed on human participants, therefore there were some ethical considerations. A consent form was provided to each participant explaining the purpose of the study and reassuring the participant that any personal information will be deleted upon the completion of the study. The microbial portion of this study did not give any ethical concern as it was conducted on their own personal stainless steel water bottles.

STATISTICAL ANALYSIS

Description of Data

The data collected for the survey was a combination of binary, multichotomous nominal and ordinal data, and numerical data (29). The first section of the survey regarded background information of the participant in which the information collected was binary and multichotomous nominal and ordinal data. The second section of the survey was a combination of nominal and ratio data as it focused on water bottle uses and cleaning practice and frequencies of the participants.

The data collected for the samples were numerical ratio data, as it used Colony Forming Units (CFUs) for bacterial levels. The plates were counted after 24 and 72 hours of incubation. The sample CFU counts ranged from zero colonies to too numerous to count. The samples that were too numerous to count were estimated to be greater than 500 CFU. The samples represent counts per 100 cm^2.

Statistical Package

The statistical software used for analysis was JMP 13 and Microsoft Excel (30,31). Data from Google Forms was exported into Excel (32). Descriptive data was analyzed and inputted into Excel. Inferential statistical analysis was done through JMP 13.

RESULTS

Descriptive Statistics

Survey

A total of 29 users participated in the survey, fitting the inclusion criteria. Gender identity, age group, and education level were asked to determine the demographics of the study (Table 1).

Table 1: Summary of Demographics

Gender identity (%)		Age Group (%)				Education Level (%)	
Male	31	*10–19 years*	0	*40–49 years*	0	*High School*	7
Female	69	*20-–29 years*	97	*50–59 years*	0	*College*	38
Other	0	*30–39 years*	3	*60+ years*	0	*University*	55
						Graduate	0

The participants were then asked questions that focused on water source and water bottle use (Tables 2). Approximately 52% (n=15) of water is sourced from the participant's home. Approximately 79% of the participants (n=23) did not use their bottles for other liquids such as juice and protein powder. Approximately 24% (each n=7) of participants refill their water bottles 1-3 times per day.

Table 2. Summary of Water Bottle Water Source and Use

Water Source (%)				Refill Frequency (%)			
Home	52	*Municipal*	0	*1X/ Day*	24	*4X/ Day*	17
BCIT Tap Water	10	*Well Water*	0	*2X/ Day*	24	*>4X/ Day*	10
BCIT Refill Station	38			*3X /Day*	24	*Unsure*	0
Other Liquids (%)							
Yes			21	*No*			79

The cleaning methods and frequency are summarized in Table 3. Soap and water was the most common cleaning method with 69% (n=20). The least common method included the sanitizing step (n=0). Approximately 59% (n=17) of the participants indicated that they generally cleaned their water bottle once a week while 24% (n=7) indicated that they had cleaned their bottle within a day.

Table 3. Summary of Cleaning Methods and Frequency

Cleaning Method (%)		Cleaning Frequency (%)			
Tap Water Rinse	24	*One Day*	24	*Within 1 Month*	3
Soap and Water	69	*Within 1 Week*	59	*Within 3 Months*	0
Soap, Water, and	3	*Within 2*	3	*Within 6*	0

Bleach		*Weeks*		*months*	
Other	3	*Within 3 Weeks*	7	*I Don't Recall*	3

Aerobic Plate Count

A total of 30 samples were plated, incubated and counted. One sample represented a control while the other 29 samples represented the participants that fit the inclusion criteria. The microbial levels of each water bottle were compared to the type of cleaning method and the cleaning frequency.

Microbial Counts and Cleaning Frequency

As there were not enough samples for all categories, data was categorized into two groups: Cleaning within 1 day (Group 1) and Cleaning within 1 month (Group 2). Group 2 included samples that were indicated to have been cleaned within one, two, and three weeks, as well as within one month. The descriptive statistics of the results are summarized in Table 4. The mean Total Bacterial Counts after 24 hours of incubation is approximately 162 CFU/100cm^2 (n=8) when the bottles were cleaned within 1 day, while bottles cleaned within 1 month is approximately 293 CFU/100cm^2 (n=22). As seen in Figure 1, the mean Total CFUs of stainless steel water bottles that were cleaned within 1 day average lower than the bottles cleaned within 1 month.

Table 4. Descriptive Statistics of Microbial Counts and Cleaning Frequency

Cleaned within 1 day				**Cleaned within 1 month**			
Bacterial Count 24 hours		***Bacterial Count 72 hours***		***Bacterial Count 24 hours***		***Bacterial Count 72 hours***	
Mean	162	*Mean*	429	*Mean*	293	*Mean*	543
Median	4.5	*Median*	202	*Median*	59	*Median*	757
Mode	0	*Mode*	1000	*Mode*	1000	*Mode*	1000
Standard Deviation	351	*Standard Deviation*	479	*Standard Deviation*	407	*Standard Deviation*	479
Range	1000	*Range*	1000	*Range*	1000	*Range*	1000
Minimum	0	*Minimum*	0	*Minimum*	0	*Minimum*	0
Maximum	1000	*Maximum*	1000	*Maximum*	1000	*Maximum*	1000
Sum	1298	*Sum*	3434	*Sum*	6435	*Sum*	11944
Count	8	*Count*	8	*Count*	22	*Count*	22

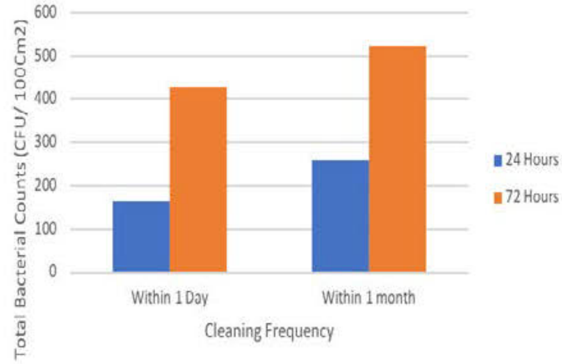

Figure 1. Cleaning Frequency and Mean CFU per 100 cm^2

Microbial Counts and Cleaning Method

As there was not enough data in for all categories, descriptive statistics was conducted only the following cleaning methods: Tap Water Rinse, and Soap and Water (Table 5). The mean Total CFU/100 cm^2 after 24 hours incubation is 234 CFU/100cm^2 (n=20) for Tap Water Rinse cleaning which was lower than Soap and Water cleaning method at 252 CFU/ 100cm^2 (n=8). The means are reversed when bacteria was counted after 72 hours of incubation with Soap and Water having the lower mean of 342 CFU/100 cm^2 (n=8) while Tap Water Rinse cleaning method was at 532 CFU/100cm^2 (n=20) (Figure 2).

Table 5. Descriptive Statistics of Microbial Counts (CFU) and Cleaning Methods

Tap Water Rinse				**Soap and Water**			
Bacterial Count 24 hours		***Bacterial Count 72 hours***		***Bacterial Count 24 hours***		***Bacterial Count 72 hours***	
Mean	234	*Mean*	532	*Mean*	252	*Mean*	342
Median	59	*Median*	608	*Median*	1.5	*Median*	104.5
Mode	2	*Mode*	1000	*Mode*	0	*Mode*	1000
Standard Deviation	351	*Standard Deviation*	483	*Standard Deviation*	462	*Standard Deviation*	441
Range	1000	*Range*	1000	*Range*	1000	*Range*	1000
Minimum	0	*Minimum*	0	*Minimum*	0	*Minimum*	0
Maximum	1000	*Maximum*	1000	*Maximum*	1000	*Maximum*	1000
Sum	4676	*Sum*	10640	*Sum*	2016	*Sum*	2738
Count	20	*Count*	20	*Count*	8	*Count*	8

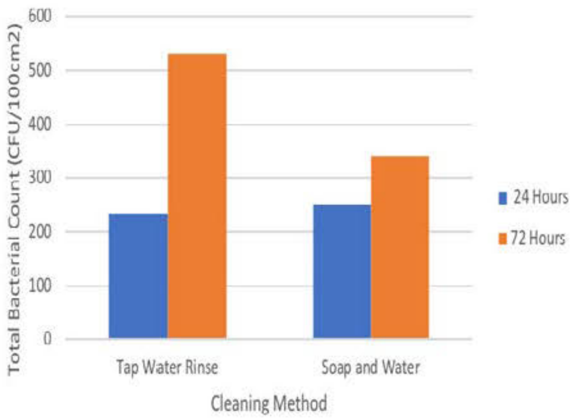

Figure 2. Cleaning Method and Mean CFU per 100 cm^2

Inferential Statistics

Aerobic Plate Count and Survey

This study compared two means of continuous numerical data, microbial counts and cleaning frequency, therefore an Independent (Two-Sample) T-test was used to compare the differences in means between Cleaning Frequency and Microbial Counts. This test was also used to compare Cleaning Methods and Microbial Counts (33).

Microbial Counts and Cleaning Frequencies Hypotheses

The following hypotheses were tested:

Ho: *There is no difference between the aerobic bacterial levels of each cleaning frequency*

Ha: *There is a difference between the aerobic bacterial levels of each cleaning frequency*

Microbial Counts and Cleaning Methods Hypotheses

The following hypotheses were tested:

Ho: *There is no difference between the aerobic bacterial levels of each cleaning method*

Ha: *There is a difference between the aerobic bacterial levels of each cleaning method*

Association Analysis of Cleaning Frequency and Cleaning Methods and Other Factors

A Chi-square test was used to determine if there was an association between cleaning frequencies and with any of the other factors analyzed in the survey such as gender, use of other liquids in the bottles, and cleaning methods. This analysis was also conducted with cleaning methods and the aforementioned factors. This test compares the frequencies or proportions within two or more groups (34).

Interpretation of Results

A summary of analysis for both Cleaning Frequency, Cleaning Methods, and Microbial Counts is shown in Table 6.

Table 6. Independent T-test Summary Results

Comparing Means	Normality	P-value	Alpha	Beta	Power
Cleaning Frequency vs. Microbial Counts	No	0.66	0.05	0.9239	0.0709
Cleaning Methods vs. Microbial Counts	No	0.6211	0.05	0.934	0.0655

Microbial Counts and Cleaning Frequencies

A Two Sample T-test was used to determine if there was variability among the means of each Cleaning Frequency. The Goodness-of-Fit Test was used to if the data was normally distributed ($p>0.05$) or not ($p<0.05$). This test determined that the data was not normally distributed for each frequency as $p=0.0003$ for Cleaned within 1 day and $p<0.001$ for Cleaned within 1 month, therefore the results from the Unequal Variances test was used. This test indicated a p-value of 0.66. The power of this test was found to be at 7.1% indicating that there was a potential beta error. To minimize this, the sample size would have to be increased. Based on the Independent T-test, there are no significant differences in bacterial levels between cleaning stainless steel water bottles daily within one month of using it. This is also illustrated through an "eyeball" test of the One-Way Analysis Vertical Plot (Figure 3).

Conclusion: *As the p-value = 0.66, the null hypothesis is not rejected. Therefore, there is no statistically significant difference between the cleaning frequencies and microbial levels in stainless steel water bottles.*

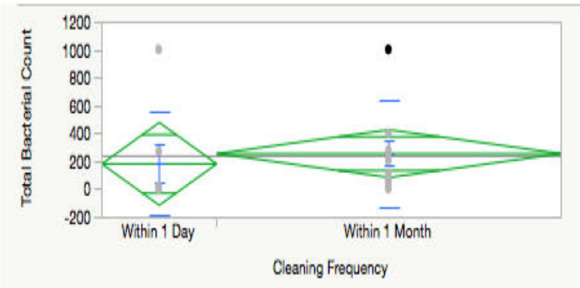

Figure 3. One-Way Analysis Vertical Plot of Cleaning Frequency and CFU levels.

Microbial Counts and Cleaning Methods

A Two Sample T-test was used to determine if there was variability among the means of each Cleaning Method. The Goodness-of-Fit Test determined that the data was not normally distributed for each method as p=0.0003 for Tap Water Rinse method and p<0.001 for Soap and Water method, therefore the results from the Unequal Variances test was used. This test indicated a p-value of 0.62. The power of this test was found to be at 6.6% indicating that there was a potential beta error. To minimize this, the sample size would have to be increased. Based on the Independent samples, T-test, there are no significant differences in bacterial levels when rinsing with water as compared to washing the water bottle with soap and water. This is also illustrated through an "eyeball" test of the One-Way Analysis Vertical Plot (Figure 4).

Conclusion: *As the p-value = 0.62, the null hypothesis is not rejected. Therefore, there is no statistically significant difference between the cleaning frequencies and microbial levels in stainless steel water bottles.*

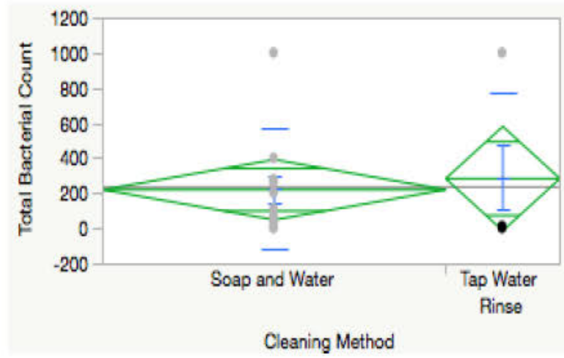

Figure 4. One-Way Analysis Vertical Plot of Cleaning Methods and CFU levels.

Association Analysis of Cleaning Frequency and Cleaning Methods and Other Factors

A summary of analysis between the Cleaning Frequency, Methods, and Other Factors are shown in Table 7 along with the p-value interpretations. For most factors tested against each other, the results indicated that the proportions were not statistically significant different from each other. The potential association between Cleaning Methods and the Use of Other Liquids is interesting as the p-value was well below the alpha level of 0.05. As this p-value is quite low, this may indicate a potential alpha error that may be minimized by adjusting the alpha level to p<0.01. If the p-value was adjusted, then there would be no potential association between the Cleaning Methods and the Use of Other Liquids. The other interesting relationship was analyzing Cleaning Frequency and the Use of other Liquids. Although the p-value was greater than the alpha level of 0.05, if was closer to it and may indicate a potential beta error. To minimize this error the sample size should be increased.

Table 7. Chi Square Summary Results

Comparing	P-Value	Interpretation
Cleaning Frequency vs. Gender Identity	0.8715	*As the p-value is greater than alpha level of 0.05, therefore the null hypothesis cannot be rejected. Therefore, there is no association between cleaning frequency and gender identity.*
Cleaning Method vs. Gender Identity	0.6646	*As the p-value is greater than alpha level of 0.05, therefore the null hypothesis cannot be rejected. Therefore, there is no association between cleaning frequency and gender identity.*
Cleaning Frequency vs. Use of Other Liquids	**0.0965***	*As the p-value is greater than alpha level of 0.05, therefore the null hypothesis cannot be rejected. Therefore, there is no association between cleaning frequency and use of other liquids* *Although, there may be a potential beta error, to minimize this error, the sample size should be increased.*
Cleaning Method vs. Use of Other Liquids	**0.0162***	*As the p-value is less than alpha level of 0.05, therefore the null hypothesis can be rejected. Therefore, there is an association between Cleaning Methods and use of other liquids.* *Although, there may be a potential alpha error. To minimize this error, the alpha level may be adjusted to a lower value.*
Cleaning Method vs. Frequency	0.9466	*As the p-value is greater than alpha level of 0.05, therefore the null hypothesis cannot be rejected. Therefore, there is no association between cleaning method and frequency.*

DISCUSSION

The microbial results show that the stainless steel water bottles that are cleaned more frequently (within one day) obtained lower levels of bacteria than those cleaned less frequently (within one month). The results also show that the bottles that were cleaned with soap and water contained lower levels of bacteria than those that were cleaned by rinsing with tap water. Although statistical analysis showed no significance, these results followed the findings demonstrated in Ouellette's study and Ryan *et al.*'s study in terms of cleaning frequency (2,22). Both of these studies show that leaving water bottles uncleaned for longer periods lead to higher bacterial counts. Interestingly, Ouellette's study found that cleaning just by rinsing with tap water obtained the lowest level of bacteria out of all the other methods (2). This differs from the results found in this study, as cleaning with soap and water showed lower levels of bacteria when compared to cleaning with a rinse.

The results of the Independent Sample T-tests for both bacterial counts obtained for cleaning frequency and cleaning methods respectively indicated that there was no significant difference. However, these results may be misleading as there was a limited and uneven sample size among the frequency and methods groups. As seen in the descriptive results, when comparing bacterial counts and cleaning frequency, the participant counts for stainless steel water bottles within one day and within a month were 8 and 22, respectively. In addition, the results for the survey may have been influenced by recall bias of the participant and/or the interviewer bias from the researcher. In terms of recall bias, the participant may not have correctly remembered when they last cleaned their water bottle and have either guessed or lied about when they last cleaned their bottle, or the method that they used. In terms of interviewer bias, the researcher when asked for clarification on cleaning frequency or method may have answered in a way that would have caused the participant to answer differently than they would have without the researcher's guidance.

Although, there was no significant difference found in the Independent Samples T-Tests for the relationship between microbial counts and cleaning frequency, the descriptive results indicated that the means of bacterial counts per 100cm^2 for bottles that were washed more than one day within a month were higher at 293 CFU/100cm^2 than those washed within a day at 162 CFU/100cm^2. This would mean that cleaning the water bottle within a day was better than washing after more than a day which was expected as cleaning more frequently reduces the bacterial load on the water bottle with each clean.

The bacterial counts were compared to PHSA's guide for Aerobic Colony Count Recommendations for Environmental Surfaces in the *Food Quality Check Program: Microbiological Recommendations* (15). The bacterial counts observed after 24 hours for the bottles that were cleaned within one day were interpreted as 'cautionary' while those cleaned within one month were interpreted as 'unsatisfactory'. This was expected as bacterial growth increases over time, which reinforces the fact that an increase in cleaning frequency is able to decrease bacterial load. The bacterial counts after 72 hours for both cleaning frequencies were interpreted as 'unsatisfactory' which was also attributed to bacterial growth. The average bacterial counts were closer together which may indicate that there is no difference between cleaning methods in reducing bacterial loads on water bottles. This result may be initially misleading, but bacterial counts after 72 hours of incubation showed a difference between the two cleaning methods even though both cleaning methods were interpreted as being 'very contaminated'. The difference between the methods was approximately 200 CFU with rinsing with tap water having the higher bacterial count at 532 CFU/ 100cm^2. This was predictive since this method does not use friction and lacks soap which has properties to dissolve and remove the bacteria and layer of biofilm (4).

The analysis of the various factors and the aforementioned water bottle user behaviors demonstrated that there was no association to each other as there was no statistical significance based on the Chi-Square Test. Although the relationships between Use of Other Liquids against cleaning methods and frequencies showed a potential significance but was found to be slightly above the alpha level of 0.05. This was found to be interesting as other liquids such as coffee or juice would require a more robust cleaning method such as using warm water, soap, and a sanitization step to effectively remove any residue left behind. Furthermore, this would have also increased the frequency required to effectively reduce the bacterial level and residue on the bottle. The lack of significance between these relationships may be attributed to the limited sample size.

To ensure that the results of this study were valid, various measures were taken. Standard tools (3M Quick Swab and Petrifilm Aerobic Count Plate) were used to test sanitation levels on surfaces, and they were used following the standard method outlined by the manufacturer (27,26). Additionally, a pilot study was performed to ensure the experiment and its methodology was conducted in a consistent manner. However, it is important to note that the results obtained may have been affected by the methodological limitations of the Aerobic Plate Count (APC). As mentioned in the literature review, the limitations of APC include not being able to identify specific disease-causing pathogens, with a low level of detection (20).

As indicated above, the sample size of this study was fairly limited, therefore, the findings of this study can only be extrapolated to the members of the BCIT community from which the samples were taken from.

Limitations

One of the largest limitations of this study was the sample size. As the sample size was quite small, and since the sample groups for each factor tested was unequal, these factors contributed to statistical beta error. As well, only members of the BCIT community were sampled from, therefore the generalizability of this study is limited to the aforementioned population. Another limitation was the timing of sample collection. Samples were not taken on certain days as they could not be counted over the weekend since the lab would have been closed, and the lab technician unavailable. Additionally, only one day of the week was allocated to work on the study. Budgetary restraints were also a limitation to this study as this resulted in fewer participants. Lastly, enumerating aerobic bacteria via APC could only provide an indication of the sanitation level of the water bottles; this method was not able to determine if there were any harmful bacteria present (20). Furthermore, counting cultures posed some difficulty due to the varying levels of culture coloring and overgrowth.

To improve the validity of this study, it is recommended that the sample size for each factor and category be increased. This would reduce the beta error and may increase the power and confidence in the study. Additionally, increasing the budget and access to the lab over the weekend would allow for a more varied and larger population to be sampled. Also, to determine if there are harmful bacteria present on the stainless steel water bottle mouthpiece, a select few cultures from the plates may be isolated.

Knowledge Translation and Recommendations

The results of this study indicated that the members of the BCIT community required improvements in personal hygiene practices as there are some who did not clean their water bottle frequently or properly. Since it would be difficult to enforce cleaning policies concerning water bottles, it should be recommended that posters be placed at water bottle refill stations around campus as reminders. These posters should include information on the importance of cleaning on a regular basis and recommended cleaning methods like cleaning with warm and soapy water with a cleaning brush. This could also be done at any other building facilities in the city or country, and should be followed through by appropriate governing bodies, such as government or corporate agencies. Another recommendation would be to install sinks that are stocked with soap and a cleaning brush, which are cleaned and stocked regularly beside the water bottle refill stations to encourage cleaning behavior before water bottle refill and use (11).

Further Research

As this study covered many aspects, this researcher recommends:

- Sampling a larger population outside of the BCIT community. This would include sampling from different municipalities, going to gyms, restaurants, community centers, schools, etc.
- Conduct a knowledge, attitudes, and practices survey regarding water bottle hygiene, associated maladies and potential pathogens to determine the knowledge level of the public
- Sample one water bottle over various cleaning frequencies with the same usage pattern and cleaning method.
- Determine if there are any harmful bacteria on the water bottle mouth pieces through isolation and PCR or any other genetic identification methods.

CONCLUSIONS

Based on the findings of this study, stainless steel water bottles are not required to be cleaned frequently as there was no statistical significance found between cleaning and bacterial load. Also, it appears that there was no significant difference among the cleaning methods. Despite the findings showing no statistical significance to support more frequent cleaning and more thorough cleaning practices, these behaviours should be encouraged to prevent and minimize the risk of potential exposure harmful pathogens. Therefore, water bottle users should minimally adhere to the recommended cleaning guidelines outlined by manufacturers such as Rubbermaid®, Contigo®, and Thermos® (8–10). As this study was limited by budget, participation, and time, further research may be required to determine if the behaviours found in this study correlate to the general public population and to see if there are any harmful bacteria present on water bottles.

Acknowledgements

The author would like to thank Helen Heacock, Fred Shaw, Keith Herle, and Jennifer Tsang for their guidance and support throughout the study.

Competing Interest

The authors declare that they have no competing interests.

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To cite this paper Vancouver Style:

Tabaco, A. (2018). Bacterial Growth in Personal Stainless-Steel Water Bottles: How Often Should You Clean Your Bottle? *BCIT Environmental Health Journal*.