An Analysis of Bacteria Colony Growth on Varying Types of Reusable Bags to Determine Ideal Conditions for Storage, Usage, and Cleaning

Abstract

Every year, over 100 billion single-use disposable polyethylene plastic grocery bags are used in the United States. Because of the negative impact they have on the environment, reusable grocery bags are now mandated in over 350 United States municipalities as an alternative to disposable polyethylene bags. This may seem like a viable solution, but reusable bags have a greater potential to become contaminated with pathogens and transmitted diseases. The purpose of this research is to determine how the amount of bacteria differs in reusable bags based on various conditions including material type, washing frequency, storage location , usage,, and the frequency of its use. To determine the amount of bacteria in each bag, Coliscan Easygel and nutrient agar plates were used. As each bag was collected, the owner completed a brief survey answering questions about the variables being tested. Bags were compared based on their identified characteristics. To analyze the data, a Student's t-tests and a single factor ANOVA test were used to determine if there was a statistical difference between the plastic and cloth reusable bags. An overall Student's t-test, one between uses, washing, and storage locations were run to determine whether or not there was a significant difference between their bacteria values. The ANOVA test was used to determine if using bags for different amounts of time has a significant effect on their bacteria amounts. Research has proven that there can be dangerous amounts of bacteria in reusable bags, which is something the population needs to be aware of. In the future, this information can be useful in analyzing health statistics and formulating a solution for the most successful method of cleaning reusable bags.

Introduction

Reusable paper bags are usually used by specialty and non-grocery stores, but reusing them is often problematic (Muthu, Li, Hu, Mok and Ding, 2012). There are a wide range of reusable bag types on the market, but the two main types are fabric reusable bags, and reusable plastic bags (**Figure 6**). All of these are heavier, more durable bags, and constructed to have a longer life ("Types of Reusable Bags", 2010). Fabric reusable bags can be made of natural fibers, such as cotton, and petroleum-based fibers, such as nylon. There are also a number of different plastic reusable bags: recycled PET reusable bag, polypropylene reusable bags, nylon reusable bags, low density polyethylene reusable bags, and conventional polyethylene reusable bags ("Types of Reusable Bags", 2010). All of these reusable plastic bags can make shopping more convenient and be better for the environment, but they can harbor copious amounts of pathogens and diseases.

The International Association for Food Protection published a study completed by the University of Arizona, which revealed that large amounts of bacteria were found in almost all of the reusable bags they tested ("Reusable Shopping Bags Could Harbor Bacteria", 2011). The study found that abundant amounts of bacteria existed on most of the bags. (Gerba, 2010) Coliform bacteria, rod-shaped bacteria used as indicators of the potential presence of pathogens, were found in half of the bags sampled (MB Laboratories, 2016). Also, the study found that 8% of the bags contained E.coli, a bacterium that occurs naturally in the intestines of people and animals, and provides protection against harmful bacteria; however, some strands of E.coli can cause food-borne infections ("Escherichia coli- Not Really That Bad", n.d.).

Another study done by the University of Pennsylvania Law school analyzes the effect of the plastic ban on the number of emergency room visits. It states that there was an increase in emergency room visits with illnesses caused by the bacteria commonly found in reusable bags in San Francisco. San Francisco was chosen for this study because it was the first place to have an official plastic bag ban. Specifically, there was a 32% increase in E.coli related admissions, a 27% increase in Campylobacter related admissions, and a 6% increase in Salmonella related admissions (Klick, 2012).

Studies have also been conducted to determine the facts that lead to an increase in bacteria in reusable bags. A study done by Arizona Commerce Authority and the University of Arizona found that only three percent of people wash their reusable bags (Williams, 2011). Washing reusable bags is an easy and efficient way to lower the amount of bacteria that all reusable bag owners should do (Klick, 2012). Furthermore, bacteria appears to grow at a faster rate if the bags are stored in car trunks. Studying the correlation between bacteria and reusable bags is important because it concerns public health, which is a top priority in today's society (Williams, 2011).

Methodology

Before Testing:

Prior to testing, collect reusable bag samples. To make transport easier, place each reusable bag into its own separate plastic bag and number it accordingly. Have the owner fill out a survey about specific variables tested in this project. Gather materials required for this project including nutrient agar, petri dishes, coliscan easygel plates and solution, and sterile cotton swabs. A sample size of 100 bags is used.

Testing Method I:

Label petri dishes with the bag number you are testing on the plate side of the dish. Heat nutrient agar in a scientific microwave until there are no solid particles left. Pour agar in petri dishes until the bottom is covered, which is usually between 10 and 15 milliliters of agar. Rotate the dish to obtain even surface coverage, and cover the dish immediately and let stand until firm. Once hardened, take a sterile cotton swab and use it to collect a sample from the bottom of each reusable bag. Then, lift the lid off the Petri dish and lightly draw a squiggly line in the agar with the end of the cotton swab you used to collect your sample. Be sure to include a plate with only distilled water and one duplicate (**Figures 11**). After all plates are complete, place them into an incubator at 35ºC for 48 hours (**Figure 7**). After that, remove plates and use a colony counter and random number generator to collect an average of the amount of bacteria per plate (**Figure 10**).

Testing Method II:

Label easygel petri dishes with the bag number you are testing on the plate side of the dish. Pour coliscan easygel mixture into each dish, and swirl plate to ensure equal coverage (**Figure 8**). Let plates sit until they are completely dry. Once hardened, take a sterile cotton swab and use it to collect a sample from the bottom of each reusable bag. Then, lift the lid off the Petri dish and lightly draw a squiggly line on the easygel with the end of the cotton swab you used to collect your sample. Be sure to include a plate with only distilled water and one duplicate (**Figures 12**). After all plates are complete, place them into an incubator at 35ºC for 48 hours. After that, remove plates and use a colony counter and random number generator to collect an average of the amount of bacteria per plate (**Figure 9**). For Coliscan Easygel plates, *E. coli* colonies will have a dark blue/indigo overall colony color, non- fecal coliforms are lighter blue/gray to purplish in color, *Salmonella* spp. appear as green/teal colonies, and *Aeromonas* spp. are pink to very light pink.

Statistics:

A Student's t-test: Two Sample Assuming Unequal Variances was used to determine if there was a statistical difference between the amount of bacteria colonies on plastic and cloth reusable bags. The same t-test was also run between bags stored in houses and cars, bags that are washed and not washed, and bags used for food items only and bags used for both food and non-food items. The alpha for all of these tests was 0.05. An ANOVA test was also run between the amount of bacteria in bags used at least once a week, at least 2-3 times a week, and at least 4-6 times a year. The alpha for this test was also 0.05.

Results

The general coliform counts for all of the reusable bags tested ranged from 0.0 colonies per 100 milliliters to 14.9 colonies per 100 milliliters (**Table 1**). A Student's t-test ran between the cloth and reusable bag types calculated a p-value of 0.2931, which is greater than the alpha value of 0.05, proving no significance between bag types. The t-test result between bags stored in houses and cars, and the single factor ANOVA test between the frequency of uses showed no statistical difference, as the p-values were 0.8010 and 0.8881 respectively. However, the p-value of the t-test between bags that were washed regularly and those that weren't was 0.0001, indicating a significant difference between that bag condition. Also, the Student's t-test between the bags used only for food and those used for all items yielded a p-value of 0.0099, showing statistical significance. The same tests were also used to compare the amount of *Aeromonas* spp. found in the reusable bags, as this was the only type of bacteria found on the Easygel plates.

However, many of the test results had no colony growth, so each statistical analysis concluded no significance.

In order to support these conclusions, bar graphs were created as a comparison of the averages between each condition (**Figures 1-5**). Standard error bars are included on each graph to account for the normal errors that occur during research. The error bars overlap on each of the graphs, except for the ones for item types and washing because they are statistically different from one another (**Figures 1-2**). The overlapping error bars on the other graphs indicate that the data is not significantly different from one another between those variables (**Figures 3-5**). Since more than three quarters of the Coliscan Easygel plates had no bacteria growth and no significance was determined, no bar graphs were made.

Discussion

From the results obtained, it can be concluded that certain variables can affect the amount of bacteria coliforms in reusable bags. Significance could not be determined between cloth and reusable bags because the p-value from the Student's t-test was 0.2931, which is greater than alpha (**Figure 5**). The same can be concluded for the t-test result between bags stored in the house and the car and the ANOVA test result between bags that are used for different frequencies of time. The p-values were 0.8010 and 0.8881, respectively (**Figures 3-4**). However, significance was determined between bags that are washed and those that are not washed. The t-test yielded a p-value of 0.0001, which is less than alpha, 0.05 (**Figure 1**). Significance was also established between bags used only for food and bags for both food and non-food items. This Student's t-test p-value was 0.0099, also less than alpha (**Figure 2**).

Some of the results of this study were expected, but some were unexpected. For example, it was unexpected that no *Escherichia coli* was found in any of the reusable bags. Typically, about 10 percent of reusable bags have *E. coli* in them at a given time (Sinclair, 2016). The significant difference between the number of bacteria colonies in the bags that are washed and those that are not washed was expected. Washing reusable bags is an easy and efficient way to lower the amount of bacteria that all reusable bag owners should do (Klick, 2012). This being said, it is reasonable that bacteria was significantly lower in bags that are washed.

Another aspect of this project was the fact that there is no significance between the bags that are stored in the house and those that are stored in the car. Bacteria usually appears to grow at a faster rate if the bags are stored in car trunks (Williams, 2011). However, this study was conducted in the winter months, when house and car temperatures are more similar to one another. Lastly, it was interesting that there was a significant difference between bacteria coliforms on reusable bags used for food items only and those used for both food and non-food items. It is reasonable that bags used for all items would contain significantly larger amounts of bacteria because they harbor all the bacteria food only bags do, along with more because of their other uses (Williams, 2011).

Many of the findings were very similar to those from other studies, but some variables were unaccounted for. The time between swabbing and testing of the reusable bags remained constant, but the time of its last use may have been a lurking variable. Also, the time of the year this study occurred may have affected the outcome because of temperature and other weather patterns. In the future, knowing there is significance between the bags that are cleaned and those that are not, it would be interesting to determine the most effective cleaning methods.

Conclusion

Ultimately, significance between bacteria amounts of varying types of reusable bags cannot be established for every variable condition, only for the amount of bacteria in bags that are washed and not washed, and in the bacteria amounts in bags that are used for only food, and both food and non-food items. Therefore, it can be concluded that certain variables affect the amount of bacteria found in the respective bags. It can accurately be determined that bags that are washed have significantly lower amounts of bacteria than those that are not washed. It can also be determined that bags only used for food contain less bacteria than those with both food and non-food uses. This information is important and the general public should be made aware of the possible dangers of shopping with these bags. Although this data may seem overwhelming, with the information in this study, the public will know the ideal conditions for usage, storage, and cleaning of reusable bags to minimize the dangers.

Acknowledgements

I would like to express my gratitude to my instructors for their support throughout my entire research process. I would also like to thank the administration for the funding required to complete my project, and my family for their continuous encouragement.

References

Greene, J. (2011, January). Life Cycle Assessment of Reusable and Single-use Plastic Bags in

California. Retrieved from https://www.researchgate.net/profile/Joseph_Greene2/publication/268297813_Life_Cycle_Assessment_of_Reusable_and_Single-use_Plastic_Bags_in_California/links/556cca2408aeccd7773be900/Life-Cycle-Assessment-of-Reusable-and-Single-use-Plastic-Bags-in-California.pdf

Klick, J., & Wright, J. D. (2013, January 4). Grocery Bag Bans and Foodborne Illness. Retrieved from https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2196481

Leverette, M. M. (2019, February 2). How to Keep reusable Grocery Bags Clean and Safe to Use. Retrieved from https://www.thespruce.com/keep-reusable-bags-clean-and-usable-2147015

Liu, Percival, Williams, Walker, Grim, Qureshi, … Drancourt. (2020). Aeromonas. Retrieved from https://www.sciencedirect.com/topics/medicine-and-dentistry/aeromonas

Loma Linda University Health. (2020). Reusable Grocery Bags. Retrieved from https://lluh.org/patients-visitors/health-wellness/live-it/online-health-show/episode-2-reusable-grocery-bags

MB Laboratories. (2016). What Does My Microbiology Report Mean. Retrieved from https://www.mblabs.com/tools-information/interpreting-your-results/explain-my-microbiology-test-results.php

MICROPIA. (2017). Escherichia coli. Retrieved from https://www.micropia.nl/en/discover/microbiology/escgerichia-coli/

MJH Life Sciences. (2011, October 12). Reusable Shopping Bags Could Harbor Bacteria. Retrieved from https://www.infectioncontroltoday.com/infections/reusable-shopping-bags-could-harbor-bacteria

Newkirk, B. (2014, January 6). Eww, reusable grocery bags' germs can make you sick. Retrieved from https://www.usatoday.com/story/news/nation/2014/01/06/reusable-grocery-bag-germs/4341739/

Parker, L. (2018, December 20). Fast facts about plastic pollution. Retrieved from https://news.nationalgeographic.com/2018/05/plastics-facts-infographics-ocean-pollution/

PennState Extension. (2016, June 6). Coliform Bacteria. Retrieved from https://extension.psu.edu/coliform-bacteria

University of Arizona. (2018). Reusable grocery bags carry E. coli. Retrieved from https://www.futurity.org/reusable-grocery-bags-carry-e-coli/

Wyckoff, W. B. (2010, June 25). Bacteria May Grow in Reusable Grocery Bags, But Don't Fret. Retrieved from https://www.npr.org/sections/health-shots/2010/06/25/128105740/plastics-industry-funded-study-finds-bacteria-in-reusable-grocery-bags

Table 1: The average amounts of general coliform growth and *Aeromonas* spp. growth (coliforms per 100 milliliters) broken down by each variable condition. The numbers for the distilled and random plates are also included. The averages ranged from 0.4 coliforms per 100 milliliters to 2.89 coliforms per 100 milliliters for the general coliform count and 0.00 coliforms per 100 milliliters to 0.39 coliforms per 100 milliliters for the *Aeromonas* spp. amounts.

Variable Condition	Average General Coliforms (coliforms per 100 mL)	Average *Aeromonas* spp. Colonies (coliforms per 100 mL)
Plastic	1.94	0.01
Cloth	2.67	0.45
Washed	0.76	0.00
Not Washed	2.67	0.26
House	2.21	0.39
Car	2.38	0.04
Food	1.31	0.01
Both	2.89	0.36
Once a week	2.38	0.00
2-3 times a month	2.32	0.02
4-6 times a year	1.91	1.41
Duplicate	0.40	0.00
Distilled	0.80	0.00

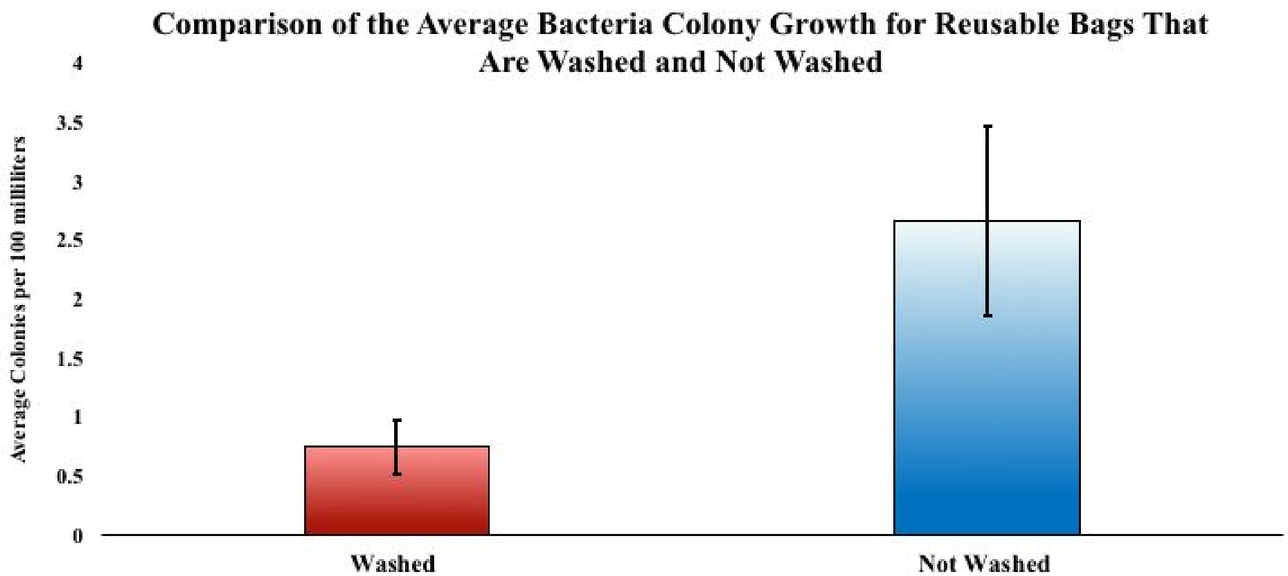

Figure 1: A comparison of the mean bacteria coliform amounts (coliforms per 100 milliliters) between reusable bags that are washed and not washed regularly. A Student's t-test yielded a p–value of 0.0001, which is less than the alpha, 0.05, indicating a statistical difference between the colony growth of bags that are washed and not washed.

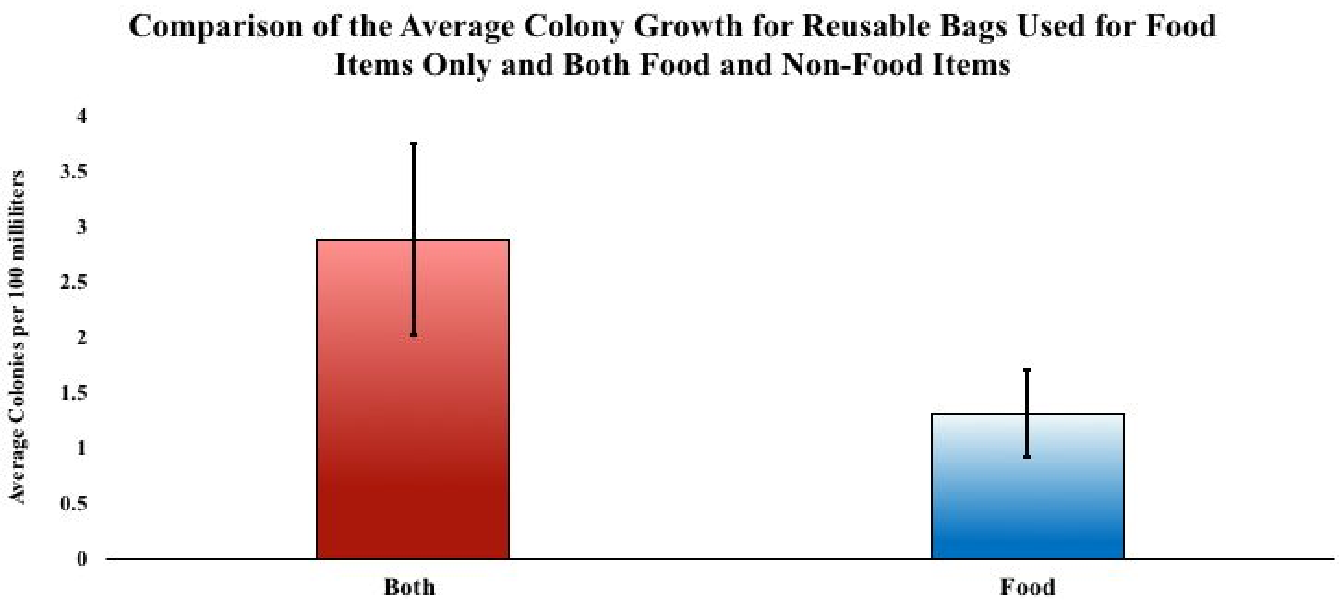

Figure 2: A comparison of the mean bacteria coliform amounts (coliforms per 100 milliliters) between reusable bags that are used for food items and both food and non-food items. A Student's t-test yielded a p–value of 0.0099, which is less than alpha, 0.05, indicating a statistical difference between the colony growth of bags that are used for food items only and both food and non-food items.

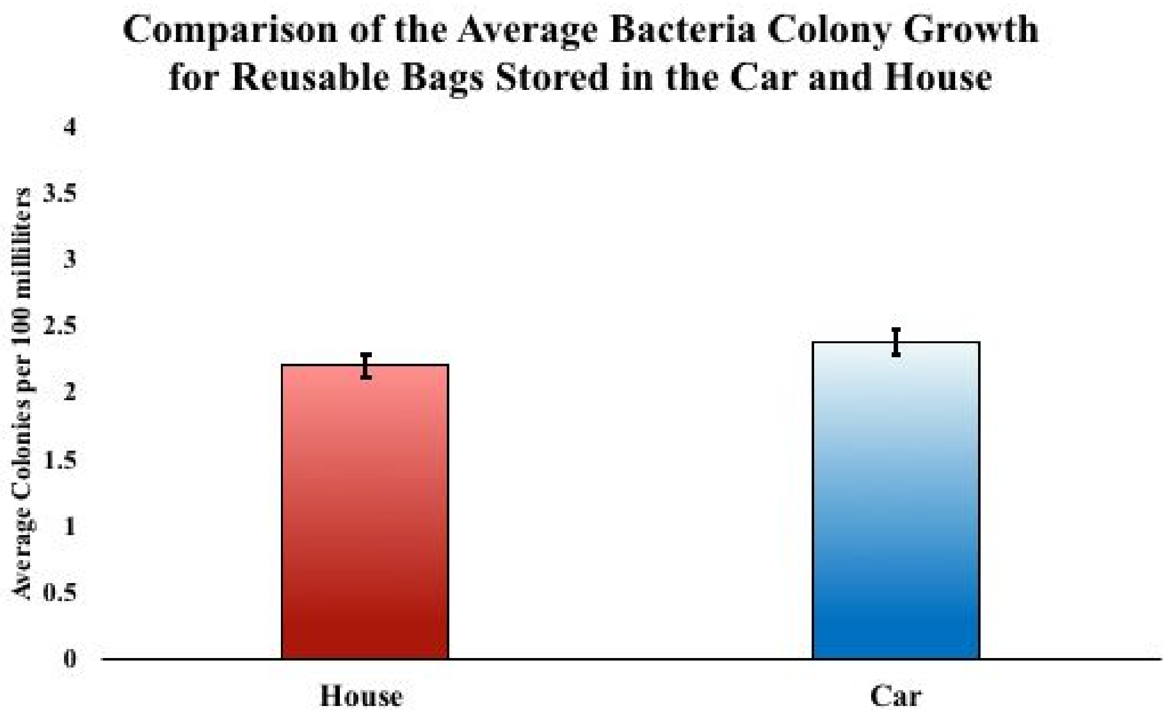

Figure 3: A comparison of the mean bacteria coliform amounts (coliforms per 100 milliliters) between reusable bags that are stored in the house and the car. A Student's t-test yielded a p–value of 0.8010, which is greater than the alpha, 0.05, indicating there is no statistical difference between the colony growth of bags that are stored in the house and the car.

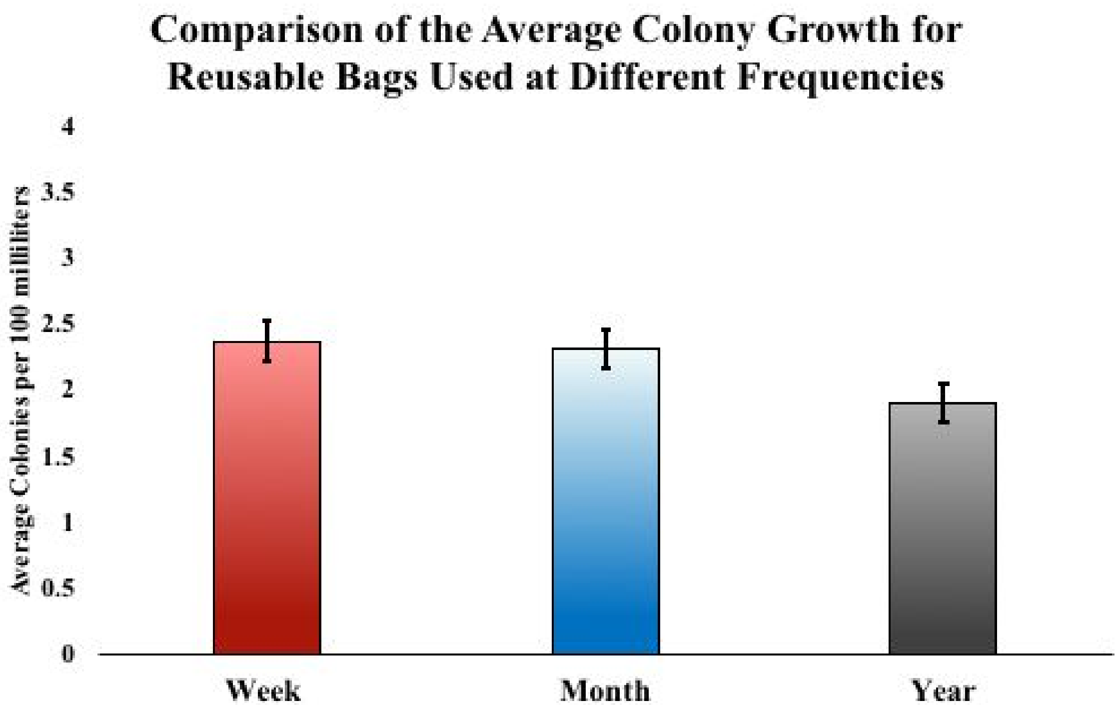

Figure 4: A comparison of the mean bacteria coliform amounts (coliforms per 100 milliliters) between reusable bags that are used for different frequencies of time. A Student's t-test yielded a p–value of 0.8881, which is greater than the alpha of 0.05, indicating there is no statistical difference between the colony growth of bags that are used for different frequencies of time.

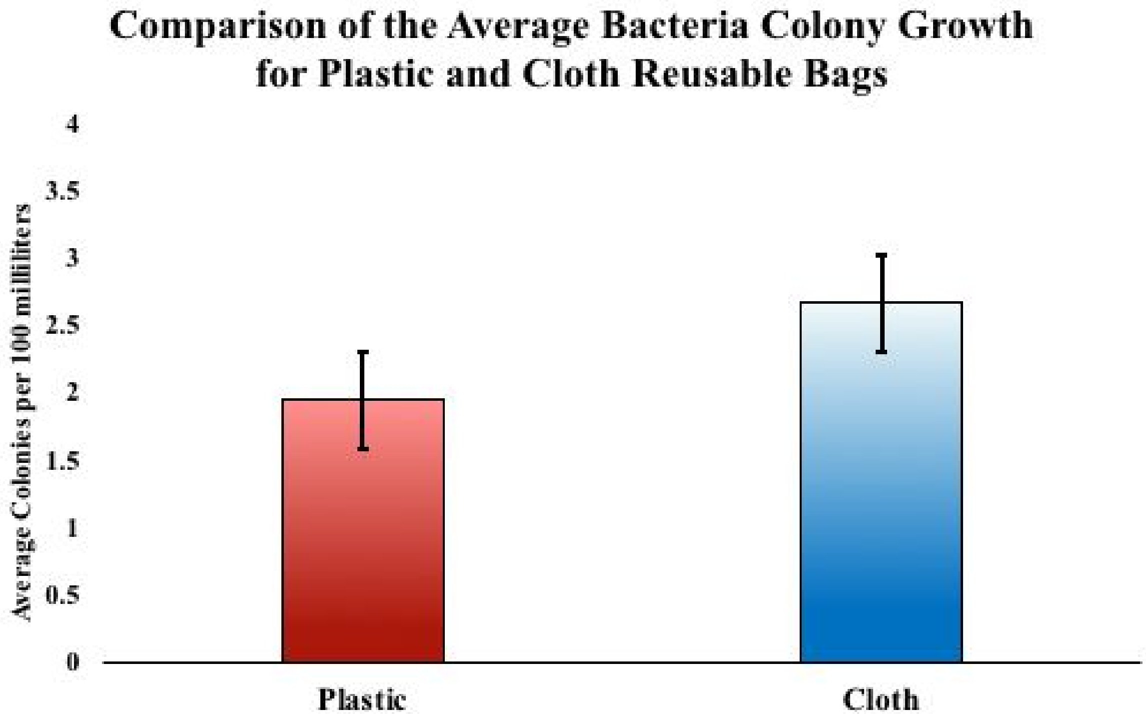

Figure 5: A comparison of the mean bacteria coliform amounts (coliforms per 100 milliliters) between reusable bags that are plastic and cloth. A Student's t-test yielded a p–value of 0.2931,which is less than the alpha, 0.05, indicating there is no statistical difference between the colony growth of bags that are made out of plastic and those that are made out of cloth.

Figure 6: An example of the reusable bags that were used for bacteria analysis. Bag type was one of the main variables examined in the study.

Figures 7-8: Research being conducted in a laboratory setting. Nutrient agar and Coliscan Easygel testing methods were used. After the petri dishes dried and bags were swabbed, plates were placed into an incubator at 35ºC.

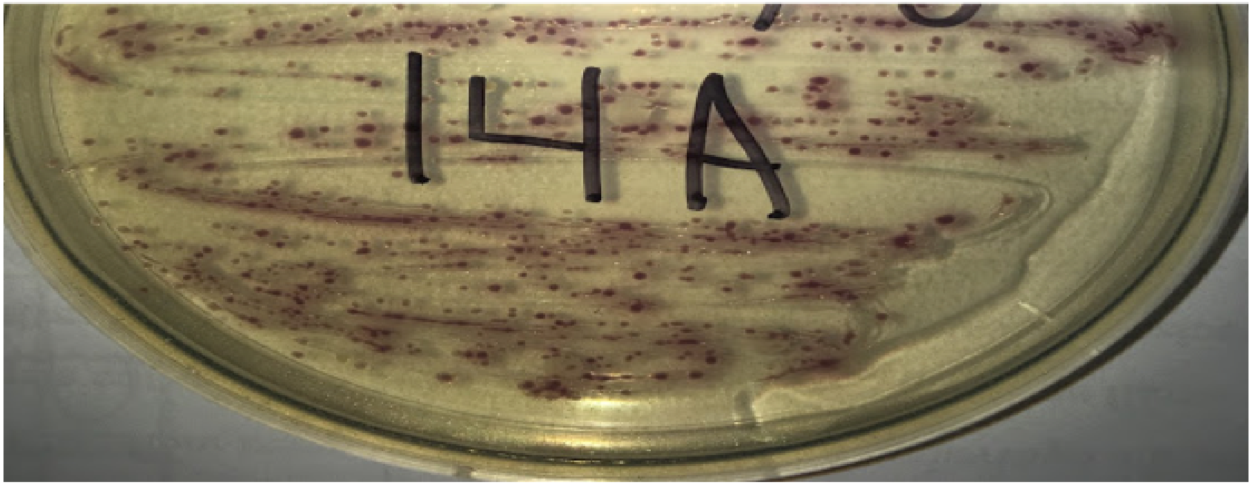

Figure 9: *Aeromonas* spp. *g*rowth on a Coliscan Easygel petri dish. *Aeromonas* spp. can cause both intestinal and extraintestinal infections, which can be severely harmful to humans.

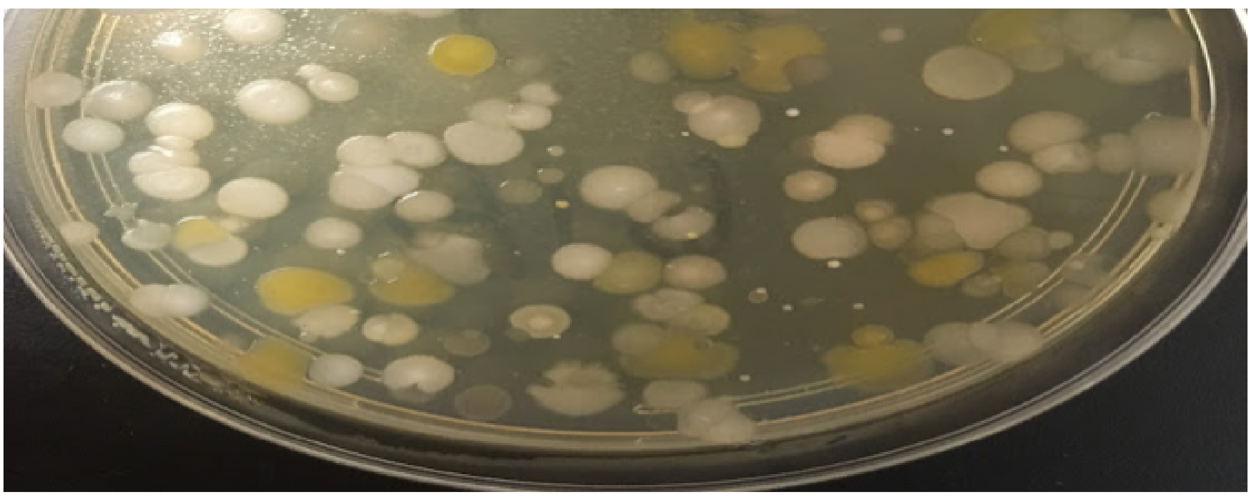

Figure 10: General coliform growth on a petri dish with nutrient agar. Coliform bacteria often are considered indicators of fecal contamination, and thus, pathogenic enteric bacteria.

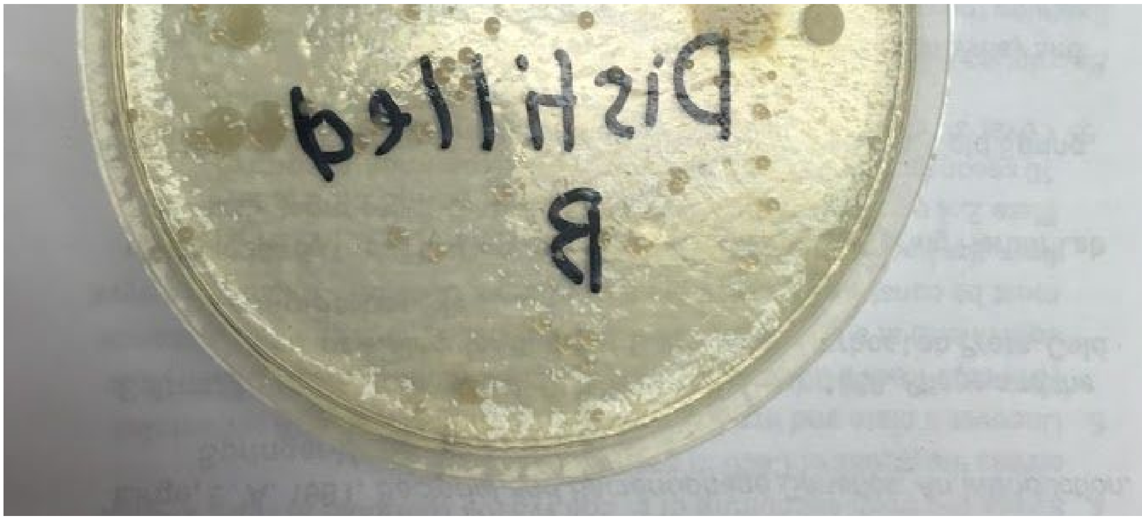

Figure 11: A petri dish with nutrient agar that only was only swabbed with distilled water. This plate is used as a comparison to other plates with the bacteria samples being tested. A random plate, or plate with no swabbing whatsoever, was also created for the petri dish with nutrient agar.

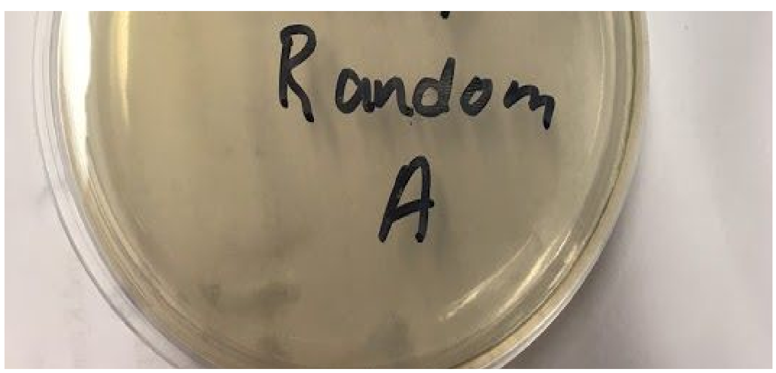

Figure 12: A Coliscan Easygel petri dish with no swabbing. This plate is used as a comparison to other plates with the bacteria samples being tested. A distilled plate, or plate with only distilled water, was also created for the Coliscan Easygel petri dish.

Appendix

Table 2: The result of the Student's T-Test run between the cloth and plastic reusable bags tested. The two-tailed p-value is greater than alpha, 0.05, indicating that there is no significant difference between these values.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	1.944	2.671111111
Variance	8.7547592	13.37846465
Observations	50	45
Hypothesized Mean Difference	0	
df	85	
t Stat	-1.0579092	
P(T<=t) one-tail	0.146547	
t Critical one-tail	1.6629785	
P(T<=t) two-tail	0.2930941	
t Critical two-tail	1.9882679	

Table 3: The result of the Student's T-Test run between the reusable bags that were washed and those that were not washed. The two-tailed p-value is less than alpha, 0.05, indicating that there is a significant difference between these values.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	0.75789474	2.67105263
Variance	1.25146199	12.6932842
Observations	19	76
Hypothesized Mean Difference	0	
df	88	
t Stat	-3.9644379	
P(T<=t) one-tail	7.4691E-05	
t Critical one-tail	1.66235403	
P(T<=t) two-tail	0.00014938	
t Critical two-tail	1.98728986	

Table 4: The result of the Student's T-Test run between the reusable bags stored in houses and cars. The two-tailed p-value is greater than alpha, 0.05, indicating that there is no significant difference between these values.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	2.20784314	2.38181818
Variance	10.3547373	11.9001268
Observations	51	44
Hypothesized Mean Difference	0	
df	89	
t Stat	-0.2528313	
P(T<=t) one-tail	0.40049081	
t Critical one-tail	1.66215533	
P(T<=t) two-tail	0.80098161	
t Critical two-tail	1.9869787	

Table 5: The result of the Student's T-Test run between the reusable bags used for food and non-food items and those used for both food and non-food items. The two-tailed p-value is less than alpha, 0.05, indicating that there is a significant difference between these values.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	2.88644068	1.30833333
Variance	14.2570544	4.21621429
Observations	59	36
Hypothesized Mean Difference	0	
df	92	
t Stat	2.63471286	
P(T<=t) one-tail	0.00494045	
t Critical one-tail	1.6615854	
P(T<=t) two-tail	0.0098809	
t Critical two-tail	1.98608632	

Table 6: The result of the Single Factor ANOVA test run between the reusable bags used at least once a week, at least 2-3 times a month, and those used at least 4-6 times a year. The p-value is greater than alpha, 0.05, indicating that there is no significant difference between these values.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.65453547	2	1.32726773	0.1188402	0.88808577	3.09543275
Within Groups	1027.50273	92	11.1685079			
Total	1030.15726	94				

2019-2020 MATES Research Project Reusable Bag Survey

Directions: Please read and answer all questions thoroughly. Be sure to answer as accurately as possible, as this will be used for scientific purposes. Please circle your answer to each multiple choice question below.

1. Where do you store this bag?
   a. House
   b. Car

2. Do you wash this bag?
   a. Yes
   b. No

3. What type of items do you use this reusable bag for?
   a. Food items only
   b. Non-food items only
   c. Both

4. How often do you use this reusable bag?
   a. At least once a week
   b. At Least 2-3 times a month
   c. At Least 4-6 times a year

For Surveyor Use Only:

Bag Number:

Date of Sampling: / /

Type of Bag: Plastic Cloth

Figure 13: A copy of the survey participants were required to fill out about each reusable bag they provided. This data was used when determining the effect different variables have on the amount of various types of bacteria in reusable bags.