2019

The Microbial Community of Kitchen Sponges: Experimental Study Investigating Bacterial Number, Resistance and Transfer

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A Thesis Submitted to Fulfill the Requirements of the
Honors Program at Assumption College

Spring 2019

Acknowledgments

I would like to express my deepest appreciation to Professor Aisling Dugan for serving as my honors advisor, mentor and role model in my life. I am incredibly grateful for her continued support and the countless number of times she has read and edited my thesis. This work would not have been possible without her. I would also like to acknowledge the Assumption College Honors Program and the Department of Biological and Physical Sciences for their support and financial assistance they provided me throughout this research. Finally, I would like to thank my parents for being there for me every step of the way through college, including this thesis. Without their love and support I would not be the person who I am today.

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Abstract

There are over 48 million episodes of foodborne illnesses in the U.S each year, 3,000 of which result in death *(CDC, 2018)*. Many of these cases may have been prevented with improved sanitation techniques. Previous studies results suggest that kitchen sponges are reservoirs for pathogens and can serve as vector of cross-contamination *(Rossi et al., 2013)*. This hypothesis proposes that the moist, porous nature of kitchen sponges not only provides microbes the perfect physical and nutritional environment, but it also provides a vehicle that allows potential pathogens to move from place to place. The goal of this 12-week research study was to quantify the bacterial number in and on typical household kitchen sponges and to test the effectiveness of common household sanitation techniques on bacterial number. In addition, the transfer of bacteria from sponge to surface was also investigated. To do this, five sponges were distributed to five participants of the Assumption College community who agreed to partake in this study. Every 7 days for a total of 28 days, a square centimeter from each sponge was removed and the bacterial load was quantified in triplicate using colony forming units (CFU) per cm^3 on nutrient agar media. Out of the 14 samples, 3 samples reached a log value $>1x10^9$ CFU/cm^3 (>9 log CFU/cm^3). Suprisingly, a significant correlation between the bacterial load and the amount of time the sponge was used was not observed as there was only a 0.4% increase in log_{10} in the bacterial abundance from week two to week three suggesting consistent bacterial loads after two weeks of use. To determine the most effective strategies for decontamination, sponges that had been used for 28 days were subjected to various treatments. Bacterial abundance was significantly reduced with the use of all sanitation methods apart from the use of hot soapy water as a cleaning technique. Bleach and ethanol treated sponges both showed a 99.9% log CFU/cm^3 decrease in bacterial growth. Lastly to test the ability sponges to transfer bacteria, a four-week

old sponge was scrubbed against a benchtop and the surface was quantified for bacterial number. The results showed an average of 5.12% bacterial transfer from sponge to surface. These data support previous studies which suggest that kitchen sponges provide a hospitable environment for microbial growth. Moreover, our data provides a clear protocol on how to properly eliminate bacterial contamination in kitchen sponges.

Introduction

Synthetic kitchen sponges are an everyday tool used to clean objects such as counter tops, cutlery, tables and dishes. In ancient times before the invention of the synthetic sponge, sea sponges were used to serve the purpose of cleaning. Their soft bodies and porous nature made them the perfect cleaning tool. However, in the 1940s the Du Pont company engineered and patented the first cellulose sponge which replaced the sea sponge in many households in the twentieth century (*How Products are Made, n.d)*. The practice of using artificial sponges to clean surfaces such as dishes, cutlery, counter top, pots and pans can still be seen in U.S. homes today and around the world. Although easier to find and cheaper to buy, artificial sponges are not ecologically beneficial to the environment. Artificial kitchen sponges contribute to deforestation and release pollutants into the air during the manufacturing process, as cellulose, one of the main ingredients in synthetic sponges, is a raw material obtained from trees. The use of sea sponges as a kitchen sponge is considered environmentally friendly as they can be sustainably harvested and that they are 100% biodegradable. A recent research study even found that natural sponges have enzymes that inhibit bacterial growth, however the mechanism of this inhibition is still unclear *(Ruocco et al., 2017)*. Although artificial kitchen sponges have now been used for decades, recent reports indicate that sponges harbor a vast number of microbes, some of which

can cause disease. Do sponges really harbor a copiousness number of microorganisms and do these germs have the potential to spread from sponge to surface, contributing to human illnesses?

According to the Center for Disease and Control Prevention, it was estimated that out of 48 million reports of foodborne illness, there were 128,000 hospitalizations and 3,000 deaths *(CDC,2018)*. This means that roughly one in six Americans will fall victim of a foodborne illness each year. It was estimated that the norovirus contributed to 58% of the illnesses, followed by nontyphoidal *Salmonella spp* (11%), *Clostridium perfringens* (10%), and *Campylobacter (9%)*. The norovirus is a more contagious virus that can be acquired by touching contaminated surfaces, coming into direct contact with an infected person, or consuming contaminated food or water with the pathogen *(CDC, 2019)*. *Salmonella spp* is a gram-negative bacterium and is the leading cause of hospitalizations in the United States at 19,000 a year *(Nordqvist, 2017)*. *Salmonella* poisoning is linked to contaminated water, fruits, vegetables and meat such as poultry and eggs and is associated with symptoms such as vomiting, diarrhea, abdominal cramps and fever.

Kitchen sponges may play a role in foodborne illnesses via cross-contamination. Consider a contaminated piece of meat being rinsed off with water in the kitchen sink before being cooked thoroughly. The high temperatures from the oven or whatever cooking method used may be high enough to kill the bacteria present in the meat however, the pathogen that was rinsed off the meat is now present in the sink or on a cutting surface. A kitchen sponge is then used to wipe down the pathogen containing surface, and then this same kitchen sponge, newly contaminated with the pathogen, could be used to clean plates and silverware, which all come into direct contact with food. This is an example of how a kitchen sponge may contribute to

cross-contaminations since previous studies have shown that they provide a hospitable environment and means of transport for bacteria.

Bacteriology

Microbes make up the vast majority of living species and are found nearly everywhere including in the ocean, soil, food and inside the human gut. A bacterium is a single-cell prokaryote that lacks membrane-bound organelles, a nucleus, and multiple chromosomes, however is still highly adaptable and complex *(Medical Microbiology, 1996)*. The most common form of bacterial reproduction is binary fission in which the cell copies its genetic material and then divides into two daughter cells. Bacteria can be classified even further into different groups based on their structure and genetic makeup. The Gram stain test is used to identify the cell wall composition of the bacteria. Gram-negative bacteria have cell walls that contain an inner membrane, a thin peptidoglycan layer in the periplasmic space and an outer membrane, while Gram-positive bacteria have a thick cell wall made up of peptidoglycan. After a Gram stain, Gram-positive bacteria will stain purple and Gram-negative bacteria will stain pink based off the structure of their cell walls. *Escherichia coli* and *Vibrio cholerae* are both examples of Gram-negative bacteria, and *Streptococcus pneumonia* and *Enterococcus faecalis* are examples of Gram-positive bacteria *(Medical Microbiology, 1996)*.

A large Gram-negative bacterial class is the *Gammaproteobacteria* class that causes foodborne diseases. This class is under the phylum *Proteobacteria*, which contains a diverse array of ecologically, medically, and pathogenic types of bacteria. It is important to mention this class of bacteria as an example since the *Gammaproteobacteria* class was seen as the most prevalent class in two studies.

Research studies that have examined bacterial content in sponges often used colony-forming unit (CFU) as a way to monitor the number of bacteria in a food source, on surfaces or within a sponge. CFU measures only culturable cells that have grown on the plate and can be useful to determine the bacterial load on a plate. In microbiology, this number is only an estimate and can be skewed, because only certain cells can grow in specific conditions, such as temperature, pH, oxygen availability, time, and media (Sutton, 2006). This is known as the plate count paradox which explains why so few colonies grow on media in laboratory conditions. Most types of bacteria are non-culturable, meaning that they are viable and present in the sample, but cannot be grown on media in the lab. For this study, the bacterial growth on nutrient agar is sufficient since it roughly demonstrates the number of heterotrophic bacteria that would grow in or on human beings.

Another method used to investigate the true number and abundance of bacteria is through the use of bacterial phylogeny and taxonomy which can be used by taking advantage of the16s rRNA gene. The 16s rRNA gene is a component of the 30S subunit ribosome and is present among all bacteria. This gene has DNA variation within it that serves as the identification marker in all bacteria which allows us to distinguish between different genera using 16s rRNA sequencing *(Janda and Abott, 2007)*. This revolutionary technique is now the standardized tool for bacterial phylogenetics which can be paired with metagenomics. Metagenomics is a new field of genetics that allows the genetic material to be sequence from a large collection of organisms without the needs to cultivate them. Both of these methods help to evaluate the true bacterial number and identify the type in any sample under investigation.

Microbiome

The microbiome is defined to be all the microorganisms that are present in an environmental community. It is estimated that in healthy humans, the ratio of microbes present in and on the body to human cells is ten to one *(Baylor College of Medicine, n.d.*). The Human Microbiome Project (HMP) was started in 2008 with the current goal to investigate the microbiome present on the human body and to analyze the role microorganism have in human health and disease (*Human Microbiome Project Consortium, 2012)*. The human microbiome consists of bacteria, viruses and single-cell eukaryotes. Although these microbes can cause sickness, some have no impact (passive riders), and some even promote health and are essential in certain body processes. Bacteria are involved in the digestion of foods, production of vitamins, and even influence the susceptibility to different chronic diseases. For example, *Lactobacillus* is a species of bacteria found in yogurts and fermented foods that produces the enzyme lactase that helps break down lactose. This type of probiotic aids in digestion and absorption of nutrients and is naturally found in the mouth, small intestines and vagina *(Hecht, 2017)*. The HMP looks further into the role of the microbiome not only within the normal human body, but also within people with different disease. Multiple research labs involved in this worldwide study additionally aim to examine if there is a difference in microbiome within people of different geographical locations, ethnicities, and diets (*Human Microbiome Project Consortium, 2012)*. This is an ongoing study funded by the National Institute of Health that is constantly receiving an influx of new information to better understand the microbial world that we live in every day.

The lack of a normal microbiome in humans can have severe effects. A study conducted at UMass Medical School Center for Microbiome Research has recently found that fecal

microbiota transplants can be used to treat *Clostridium difficile* (*C.diff)* infections *(Pellish, n.d)*. *C. diff* infections are most commonly seen in patients in hospitals, since the use of antibiotics kills the normal microbiome and allows the resistant *C. diff* to overpopulate and outcompete the normal microbiome in immunocompromised people, permitting *C. diff* to colonize the gut. *C. diff* causes inflammation of the large intestines which can cause the victim to suffer from diarrhea, bloody stool, fevers, abdominal cramps and dehydration *(Nordqvist, 2017)*. Although it is rare, this infection can lead to death, especially in elderly patients *(Nordqvist, 2017)*. The use of a fecal microbiota transplant involves stool from a healthy donor being placed inside the colon of someone infected with *C. diff* infections to reintroduce a normal microbiota to the infected individual. The first course of therapy is still metronidazole and vancomycin to treat this infection, however as of last year, The Infectious Disease Society of America issued new guidelines, recommending the use of fecal microbiome transplantations for patients who suffer from recurrences of *C. diff* infections. This is currently an investigational treatment, but under the FDA providers are allowed to perform this procedure on patients who have failed the antibiotic treatment and continue to suffer from *C. diff infections (Cooney, 2018*). The results from the UMass research have shown that the symptoms of *C. diff* infections resolved in 90% of patients who underwent this transplant and were less susceptible to recurrence of *C.diff* infections *(Pellish, n.d)*.

The field of microbial biogeography examines all of the microbes that live in a given environment. One study investigated the microbial communities in built environments of ten houses in Checherta (traditional jungle village), Puerto Almendras (rural village), Iquitos (large Peruvian village) and Manaus (city in Brazil), by taking bacterial swabs of the walls and floors in these houses *(Claderon et al., 2016)*. The researchers found that the participant's homes in

Checherta and Puerto Almendras were covered with microbes from the outside environment, while houses in Manaus and Iquitos were contaminated with microbes that most likely came from humans. No significant differences were found across urbanized homes, but the researchers found a pattern between the microbial community and the type of room it was. For example, kitchens were contaminated with bacteria that are mostly found in water sources and bathrooms were contaminated with microbes that are found in the mouth *(Claderon et al., 2016*). The researches concluded that urbanized spaces showed an increase in human-associated microbes compared to environmental microbes, increasing the risk of the transmission of potential pathogens *(Claderon et al., 2016*).

Investigating bacteria in Kitchen sponges

A study performed by the National Sanitation Foundation in 2011 investigated the number of coliform bacteria in common kitchen surfaces. Coliform are gram negative, rod shaped bacteria of fecal origin. This study showed that 75% of sponges and dishcloths tested in 22 households were found to contain coliform bacteria *(NSF RSS, 2011)*. Are sponges harmful and does using them outweigh the benefits they provide? This manuscript will report our research that explores bacterial number in kitchen sponges and ask how used kitchen sponges can be cleaned and whether they provide a means of cross-contamination.

A study in Jimma, Ethiopia observed that kitchens sponges are heavily contaminated with bacteria *(Wolde & Bacha, 2016)*. 201 kitchen sponges were collected from various locations including restaurants, hotels, cafeterias and pastry shops. A 225mm^3 piece of sponge was aseptically removed, diluted in peptone water and plated on a variety of agar plates followed by incubation at 32^{o}C for 48 hours. Their research revealed that 64.9% of the sponges surveyed

contained an average coliform count of one billion CFU/cm^3. The presence of coliform bacteria is an indicator of fecal contamination and potential pathogens. Although not every coliform bacterium will cause illness, it serves as an indication that there could be contaminants present. Some notorious coliform species that cause disease at low doses are *E.coli* and *Shigella*, however most strains of these bacteria are unlikely to cause illness. Strikingly a coliform count as high as 630 billion CFU/cm^3 was seen in one sponge from a pastry shop in Jimma *(Wolde & Bacha, 2016)*. Using different cell morphology tests such as catalase test, cytochrome oxidase test and Gram staining on bacterial colonies, it appeared that the genus of bacteria most prevalent was *Pseudomonas,* belonging to the *Gammaproteobacteri*a class. *Bacillus, Micrococcus, Streptococcus* and *Lactobacillus* groups were also commonly found in these kitchen sponges *(Wolde & Bacha, 2006).*

A different study using 16s rRNA sequencing conducted in Germany by Cardinale *et al.,* also showed that the class *Gammaproteobacteria* (51.1%) was seen to be the most prevalent subphylum of bacteria found in the microbiome of used kitchen sponges. 33,181 high quality DNA sequences were analyzed from the 28 sponge samples and 362 taxonomic groups were formed (97% sequence similarity was the threshold) to identify the genera from these samples *(Cardinale et al, 2017).* This study also utilized 16s rRNA classification which was coupled with a technique called, fluorescence in situ hybridization (FISH) and confocal microscopy to analyze the microbiome of the sponge. FISH provides a way to visualize and map the genetic material in a cell. It can be used to identify where genes appear on certain chromosomes. In this study, the researchers used FISH to track bacterial DNA and were able to see the arrangement patterns of the bacteria in kitchen sponges *(Cardinale et al, 2017).* The results suggested that

bacterial colonization mostly appeared on the surface of the sponge and was able to create a biofilm-like structure (*Cardinale et al., 2017).*

Sanitizing Sponges

Several studies agree that vast numbers of bacteria accumulate in the kitchen sponge *(Cardinale 2017, Wolde, 2016* and *Sharma, 2009)*, however there is little agreement on the proper way to clean sponges to reduce the maximum amount of bacteria. One study asked how sponges should be sanitized to reduce bacterial growth and how often should these cleaning techniques take place. This study investigated the variety and spatial arrangement of microbes within kitchen sponges that were treated with heat using a microwave or hot, soapy water *(Cardinale et al., 2017)*. Researchers observed little difference in bacterial count between these "special cleaned" and uncleaned sponges *(Cardinale et al., 2017)*. Their data showed that the treated sponges did not show a significant difference in bacterial load in treated versus non-sanitized sponges and in fact, indicated that this type of cleaning increased the abundance of two particular genera, *Moraxella* and *Chryseobacterium* by 20% and 15% respectively *(Cardinale et al., 2017)*. These two genera are members of risk group 2 related bacteria which are microorganisms that cause disease in humans, although the diseases are treatable and preventable. Risk 2 species includes bacteria in the *Streptococcus* genus and viruses of the Herpesvirus family. *Chryseobacterium* is categorized in the Risk 2 and, is a gram negative, non-spore-forming, rod shaped bacteria seen in raw meat and milk *(Dugas et al., 2001).* *Chryseobacterium mesingoseptica* is involved in serious infections such as neonatal meningitis which can result in death without the proper treatment *(Tesini, 2018)*. The *Moraxellaceae* family is commonly found on human skin, however certain species such as *Moraxella catarrhalis* can

cause ear infections, lower and upper respiratory infections and sinusitis in people *(Bush, and Perez, 2018)*. These resistant bacteria were believed to have survived the sanitation process and rapidly re-colonized the sponge. Therefore, these data suggest that the sanitization of sponges may promote a higher number of Risk 2-related species *(Cardinale et al, 2017)*. One problem of this study was that the two variables concerning special cleaning (hot, soapy water or microwave treatment) were not differentiated. In other words, their data made it unclear on which sanitation method, if not both, increased RG2 species.

The conclusions drawn from this study were seen very controversial. Quinlan, a food biologist from the Department of Nutrition Sciences at Drexel University, directly contradicted Cardinale *et al* results by publicly stating "We do not want to make public health recommendations based on five sponges from Germany" (*Doucleff, 2017)*. Quinlan believes that disinfecting sponges with "hot soapy water" would actually encourage the growth of bacteria and does not believe it should have been a sanitation method used in the Cardinale *et al*. findings. Quinlan and her colleagues conducted a research study on 100 households in Philadelphia and found that 64% of the homes that were investigated had fecal coliforms in the kitchen sponges with an average of 41,686 CFU per cm^3 (Table I) (*Borrusso & Quinlan, 2017)*. Although Quinlan did not test the use of sanitation methods on sponges, she agrees with the U.S. Department of Agriculture (USDA) instructions for sponge sanitization which recommends washing sponges using a standard dishwasher or placing wet sponges in the microwave for a minute every day to kill most pathogens. Based on data from the USDA, these methods are believed to decrease the number of bacteria by a million-fold, while targeting the most dangerous microorganisms with the extreme heat *(Sharma et al., 2009)*. The USDA found that microwaving used sponges showed a 99.9999% reduction of bacteria, while sponges placed in the dishwasher

showed an equally promising 99.9998% reduction of bacteria after treatment *(Sharma et al., 2009).*

Although not a significant amount of information is found that warrants Quinan's claim that hot-soapy water leads to an increase in bacterial load, previous studies have found that placing sponges in boiling water significantly reduced the bacterial load *(Tate, 2006 and Rossi et al., 2012)*. In a study by Tate *et* al., 48 kitchen sponges that were used for two weeks were collected and cleaned using different types of sanitation methods. Tate saw a 47.2% reduction in bacteria number after the sponges were boiled for ten minutes compared to the untreated sponges in his study *(Tate, 2006)*. Rossi et al. study, found that boiling used sponges that harbored an average bacterial abundance of 1,258,925,411 CFU/sponge (Table I), appeared to have a 99.9999% bacterial load reduction *(Rossi et al., 2012)*. In the prior study conducted by Cardinale *et al.*, the temperature of the hot, soapy water was not recorded which makes it difficult to compare the similarity of these two types of cleaning techniques. The recovery time for the sponges was not noted for both studies either which also may contribute to the discrepancies between the studies.

An additional source commented that washing sponges with warm soapy water may decrease bacterial contamination, but the soap may stay in the sponge and lead to soap scum, however there is no data shown or given that proves this hypothesis *(Troy, Eric, 2014)*. Soap scum results from a combination of calcium and magnesium particles found in the water with soap to form a whitish gray film over a surface. Although it is not harmful, it can contribute an increase in bacterial number as bacteria can live and rapidly colonize in the scum *(Recer, 2014)*.

Another study conducted by Sharma and colleagues analyzed the effectiveness of chemical treatments on sponges that were artificially inoculated with bacteria in Baltimore, Maryland *(Sharma et al., 2009)*. In their study an unspecified amount of kitchen sponges were

mixed with 1300 ml of tryptic soy broth containing lean (90%) ground beef and left at 22° C for 48 hours. The sponges were then either cleaned with deionized water, lemon juice (pH 2.9), 10% solution Clorox, microwaved, or dishwashed. Their study's findings supported Quinlan's suggestion that the best method to sanitize sponges are in the microwave. The results showed that microwaving the sponges was the most effective method in killing the bacteria, having a CFU count of only 3 CFU/sponge compared to the untreated control that had bacterial count of 31,622,776 CFU/sponge *(Sharma et al., 2009*. Dishwashing was more effective than the bleach and lemon juice method, with only 63 CFU/sponge bacteria surviving the dishwashing treatment. As seen in Table 2, the chemical treatments and tap water treatment showed high bacterial survival similar to the untreated sponge *(Sharma et al., 2009).*

It was proposed that the ineffectiveness of the bleach was due to the sodium hypochlorite found in the bleach which may have become inactivated due the amount of organic soils present in the sponge derived from the meat (*Kotula, et al, 1997).* Another hypothesis is that bacterial and fungal cells adhere to the surface of the sponge and form a biofilm that prevents the hypochlorite in the bleach from penetrating and killing the bacteria found within the interior of the sponge *(Ryu & Beuchat, 2005).* According to an experimental study, planktonic bacteria such as *Staphylococci, Pseudomonas and E.coli* can form and evolve a full biofilm within two to four days if the environment and population number is favorable. Bacteria that form biofilms can become more tolerant to disinfectants and antiseptics (*Phillips et al., 2010)*. The formation of a biofilm was also observed in Cardinale *et* al study by using FISH and confocal microscopy on one week to one month used sponges (unspecified). From the spatial arrangement images, it appeared that bacterial colonization occurred mostly on the surface of the sponge, indicating a biofilm within the internal cavity walls. *(Cardinale et al, 2017).*

Can Sponges Transfer bacteria?

Many studies have demonstrated that sponges harbor vast quantities of bacteria although consensus on how to sanitize these sponges is lacking. Another important question is whether bacteria effectively transfers from a sponge to a surface. Donofrio *et al., 2012 and* Eliandra Rossi *et al., 2013,* both studied the transfer of microorganisms from sponge to surface. Donofrio *et al.* found that cleaning frequency and type of cleaning (aggressively scrubbed or lightly wiped) had a significant effect on bacterial transfer. The researchers found that more aggressive cleaning methods did liberate higher numbers of bacteria to the surfaces, therefore increasing the amount of bacteria transferred from sponge to surface *(Donofrio et al., 2012).*
Eliandra Rossi *et al.*, investigated if there was any type of surface that would promote the transfer of bacteria. Their results showed that 21%-43% of bacteria present in sponges can be transferred to a new surface, however the number of microbes transferred to the surface was not dependent not on the surface type but was highly dependent on the initial contamination of the sponge *(Donofrio et al., 2012).* In other words, sponges with a greater number of bacteria transferred more bacteria to surfaces. The average sponge in their study showed a CFU count of 6,309,573 CFU/cm^2 per sponge and the transfer of bacteria from the sponges to the stainless steel ranged from 1,995-316,227 CFU/cm^2 *(Rossi et al, 2013).*

The FDA does suggest that specific species of bacteria like *Staphylococcus aureus* and *Bacillus cereus* found at levels greater than or equal to 10,000 CFU/g in food to be considered adulterated, meaning of poor quality. These are non-binding recommendations (not a definitive answer) from the FDA, however this gives a sense of how much and what kinds of bacteria count is considered too much and unsafe. Wolde and Bacha study showed that 98.7% of their sponges

had a bacterial count greater than 10,000,000,000 CFU/cm^3 (Table I), 1,000,000 times the amount of what is to be considered contaminated. Although sponges are not consumed, sponges can act as disseminators of pathogens and can transfer bacteria to plate and utensils leading to cross-contamination.

Many strains of *Staphylococcus* are harmless, however, there are some species that can be found in dairy products that produce enterotoxin. *Staphylococcus aureus* enterotoxins (SEs) when ingested enter the gastrointestinal tract and cause the victim to experience symptoms such as nausea, vomiting, cramping, transient change in blood pressure, pulse rate and many other undesirable symptoms. This toxin is not inactivated at high temperatures, when the milk is pasteurized *(U.S. Department of Health and Human Services. Food and Drug Administration, 2009)*. Studies have shown that SEs have the ability to penetrate the gut lining in humans causing sepsis and toxic shock like symptoms. A certain type of toxin produced by SEs, was seen as the most common enterotoxin present in foodborne outbreaks in the US (77.8% of all outbreaks) (*Argudin et al., 2010)*. The SEA contaminated food items were mostly processed meat and dairy products that were improperly handled and stored at elevated temperature (*Argudin et al., 2010)*. Symptoms of this foodborne illness caused by SEA are rapid onset nausea, vomiting and diarrhea and depending on the severity may require hospitalization (*Argudin et al., 2010)*.

Summary

The studies explored all agree that kitchen sponges harbor a vast amount of microbial life. Although taking precautions against certain harmful strains of bacteria are extremely important, we should be aware that most bacteria have either little or no harmful effects. Bacteria present in the human gastrointestinal tract helps break down food and probiotics in yogurt, milk and cheese promote a healthy immune system and digestive tract. Microbes can also be used to clean up oil spills and help turn raw sewage water into clean water. Surprisingly, humans have ten times more microbial cells than human cells. That means that there are around 100 trillion bacterial cells to the 900 trillion bacterial cells in the average human body, so there is no way to escape them (*National Institutes of Health, 2012).*

Kitchen sponges do not pose an immediate threat since most people will not fall ill if an unwanted pathogen comes into contact with their food. However, those who are immunocompromised such as newborns, people with HIV/AIDs, cancer and transplant patients, pregnant mothers, those on immunosuppressant therapy and others who have other illnesses and diseases may be at extreme risk. Patients who are immunocompromised have a reduced ability to fight infection compared to healthy people. B-cell and T-cell defects make these patients predisposed to serious infection from common pathogens such as *S. pneumoniae, P. aeruginosa, Legionella pneumophila, L monocytogenes, Nocardia* species, *Mycobacterium* species *(Schreier et al., 2015).*

In a healthy person the immune system is there to protect the body from foreign invaders by creating a barrier that does not allow bacteria to enter the body. The epidermis contains Langerhans cells, a type of dendritic cells that play a large role in the immune system by being the first line of defense by eliciting a signal to T-cells. Many people with immunodeficiency such

as the HIV disease experience a lower amount of langerhan cells *(Jaitley, 2012)*. This means that if a pathogen comes in direct contact with someone with immunodeficiency syndrome, they may have a much harder time defending off the foreign invaders.

This study aims to educate people about the potential sources of contamination in the kitchen. Most bacteria found in homes and in kitchen sponges are not dangerous. However, people should take precautionary measures by practicing sanitation methods in the rare event that some dangerous microorganism is introduced into the kitchen. This will help ensure that microbes doe not come into direct contact with their food, kitchen surfaces and utensils. Therefore, it is important to thoroughly and efficiently clean all dishware, appliances, sponges, and the kitchen surfaces. Understanding the sanitization techniques that work effectively and consistently to kill bacteria and establishing an efficacious protocol is critical to preventing the spread of infections in food process facilities, restaurants, and at home.

There were three major aims of this research study. The first aim was to quantify the bacterial load found in used kitchen sponges, and to see if there were any correlations between bacteria abundance over time used. The second aim was to test different sanitation techniques and provide experiment evidence to determine proper ways to clean kitchen sponges. The last aim of this project was to investigate the role kitchen sponges have as vehicles spreading bacteria or transferring bacteria. Together, these results from this research study gave us a better understanding of the true bacterial contamination in used kitchens sponges and the steps needed to take to ensure proper sanitation in the kitchen area.

Methods

Quantification of Bacteria in Kitchen Sponges over time

Five new ScotchBrite delicate care scrub sponges were distributed to five participants who are employed at Assumption College in Worcester, MA. Participants were asked to use the sponges in their kitchens as they normally would for a four-week period and to return them back to Assumption College every seven days for a total of 28 days. At that time, approximately one-eighth of the sponge was removed before returning the sponge back to the participants. Next, one cubic centimeter piece from each of the sponges and a non-used sponge (control) were aseptically removed using a sterile blade and then separately added to 1.0mL of nutrient broth (NB). This step was conducted in triplicate for each participant's sponge. After vortexing in nutrient broth 20 seconds, a 10-fold serial dilution series was then performed for each sample. 100 ul of diluted NB was spread onto Criterion Nutrient Agar (NA). The plates were incubated at 32^{o} C for 48+/ 4 hours. The bacterial load for each plate was quantified using colony forming units (CFU) per cm^{3} and log CFU/sponge.

Sanitation Techniques in Sponges

In duplicate, each of the five sponges that had been used for 28 days were cut into one cubic centimeter pieces and subjected to one of the five different sanitation treatments or no treatment. The sanitation conditions were as followed: (1) microwave oven on high for one minute, (2) dishwasher on heavy cycle, or fully immersed for one minute into a sterile beaker containing 250 ml of either (3) hot soapy water (55^{o} C), (4) 10% solution of household bleach, or (5) 70% ethanol. Sponge pieces treated by the microwave oven were soaked in distilled water

and then placed on the turntable of a household microwave oven on full power for one minute. These pieces were then immediately transferred to 1.0 ml of NB. Sponge pieces treated with the dishwasher were placed on the top rack of a basic household dishwasher and treated under a heavy heat drying cycle using Cascade Dishwasher Detergent, Fresh Scent ActionPacs. These pieces were transferred to 1.0 ml of NB, 1-5 hours after the cycle ended. For conditions 3-5 the sponge pieces were washed with distilled water after treatment, before being transferred to 1.0 ml of NB. In addition, there was an untreated control for each 28 day used sponges. Once sanitized, each of the samples along with the control, underwent a 10-fold serial dilution and 100 ul of the various dilutions were spread onto NA before incubation at 37^{o}C for 48+/ 4 hrs. CFU were scored to determine CFU/cm^3 for each condition and the control.

Transfer of Bacteria from Sponge to Counter Surface

To investigate the transfer of bacteria from a sponge to counter surface, one four-week regularly used kitchen sponge was studied. Three 10cmx10cm squares were marked off with tape on an unsanitized epoxy resin top lab bench. In the first 10x10cm^2 lab bench, the used sponge was soaked in dH_2O and rubbed on the bench inside the taped off area for ten seconds. The sponge was scrubbed onto the surface the way a sponge would be used to normally clean a kitchen counter, allowing about 1/3 of the sponge to make direct contact with the surface. On the second 10x10cm^2 taped region, the same process was repeated with a brand-new clean sponge as the negative control. In the third 10x10cm^3 region, the lab bench surface was left untouched and no sponge was used. The swab method was then used to quantify the enumeration of bacteria transferred to the surface. A sterile cotton swab was soaked in sterile dH_2O and used to briefly swab the entire 10x10 cm^2 marked off surface for each of the taped off surfaces. Each

inoculated cotton swab was then transferred into 1.0 mL of NB and agitated to release the microbes present on the swab into the solution. The samples were vortexed in NB for 20 seconds before a 10-fold dilution series was conducted. 100 ul of each dilution was plated on NA and the plates were incubated at 37° C for 48+/ 4 hrs., counted and expressed as log CFU/sponge. This experiment was conducted in duplicate. A schematic overview of the experimental design is outlined in Figure 1.

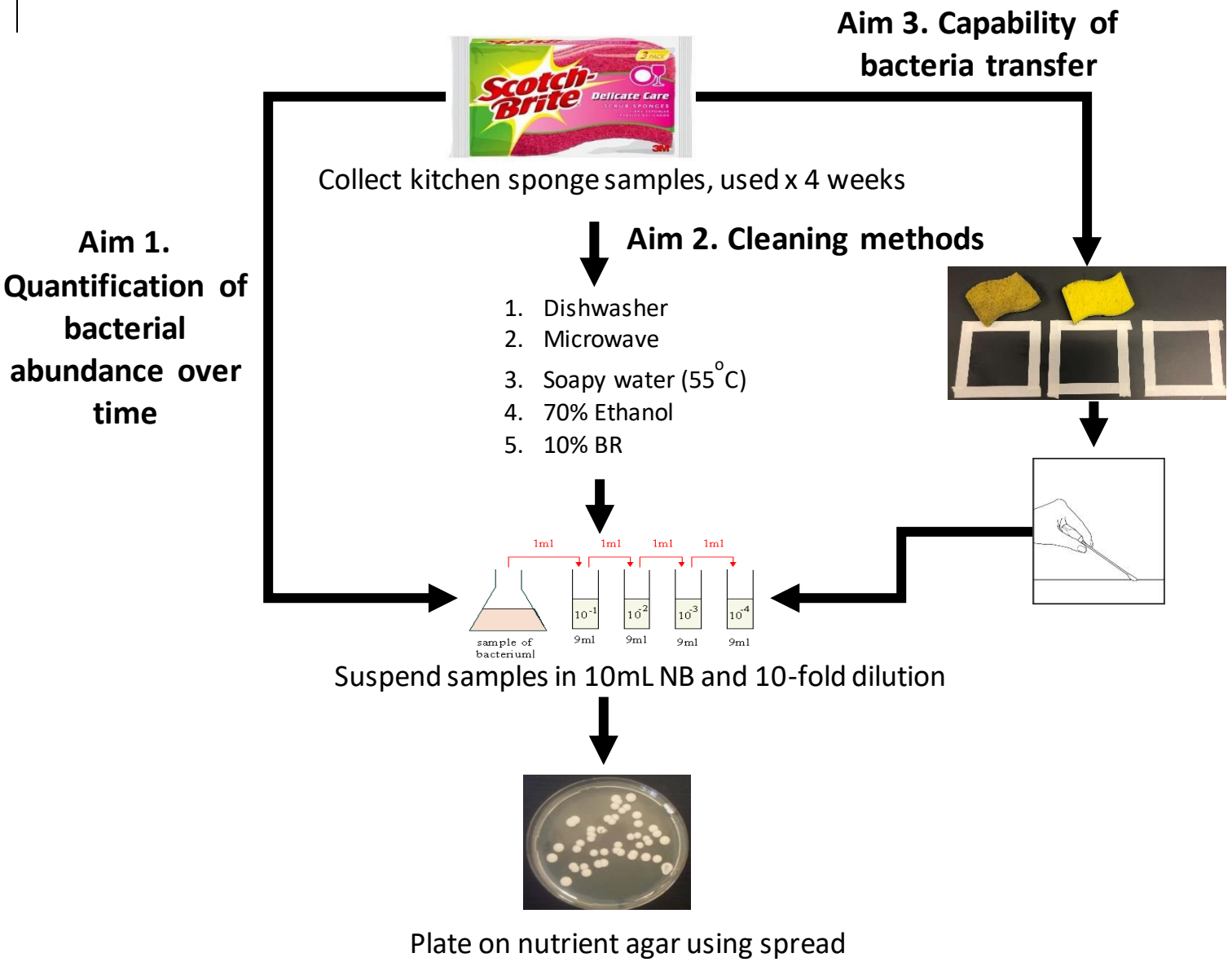

Figure 1. Experimental setup of the three-part procedure, investigating bacterial abundance over time, effectiveness of cleaning methods on bacteria, and the ability of sponges to acts as a vehicle for bacteria.

Results

The goal of this 12-week study was to quantify the bacterial number in household kitchen sponges, test the effectiveness of household cleaners and to investigate the potential bacterial transfer capability of kitchen sponges. The data showed that 1) bacterial contamination in sponges as high as 10^9 CFU/cm^3 , 2) chemical methods reduced contaminated sponges up to 99.9%, and 3) an average of 5.12% of bacteria can transfer from sponge to surface.

Investigating Bacteria in Kitchen Sponges Over Time

To quantify the number of bacteria that accumulate in kitchen sponges over a four-week period, sponges were distributed to five participants. Participants were told to use their sponges in the kitchen as they normally would, and to return the sponges every seven days for four weeks. Each week one square centimeter was aseptically removed, placed in 10mL of nutrient broth and serially diluted. This was done in triplicate, so there were three sponge samples per week from each participant's sponge. The results from each week are displayed as an average of the three samples (Figure 2). A representative image of the abundance of bacterial retrieval at two, three and four weeks shown in Figure 3. The yellow spots on the NA plates are the visual appearance of bacterial colonies were used to estimates the number of bacteria in the used sponges for each sample throughout the four-week study. To determine the total number of bacteria in each cm^3 of the sponge, the following calculation was used: #bacterial colonies / (dilution x volume). The results of this study are expressed as log CFU/cm^3 in Table I. The CFU count for week one was not included due contamination in the lab.

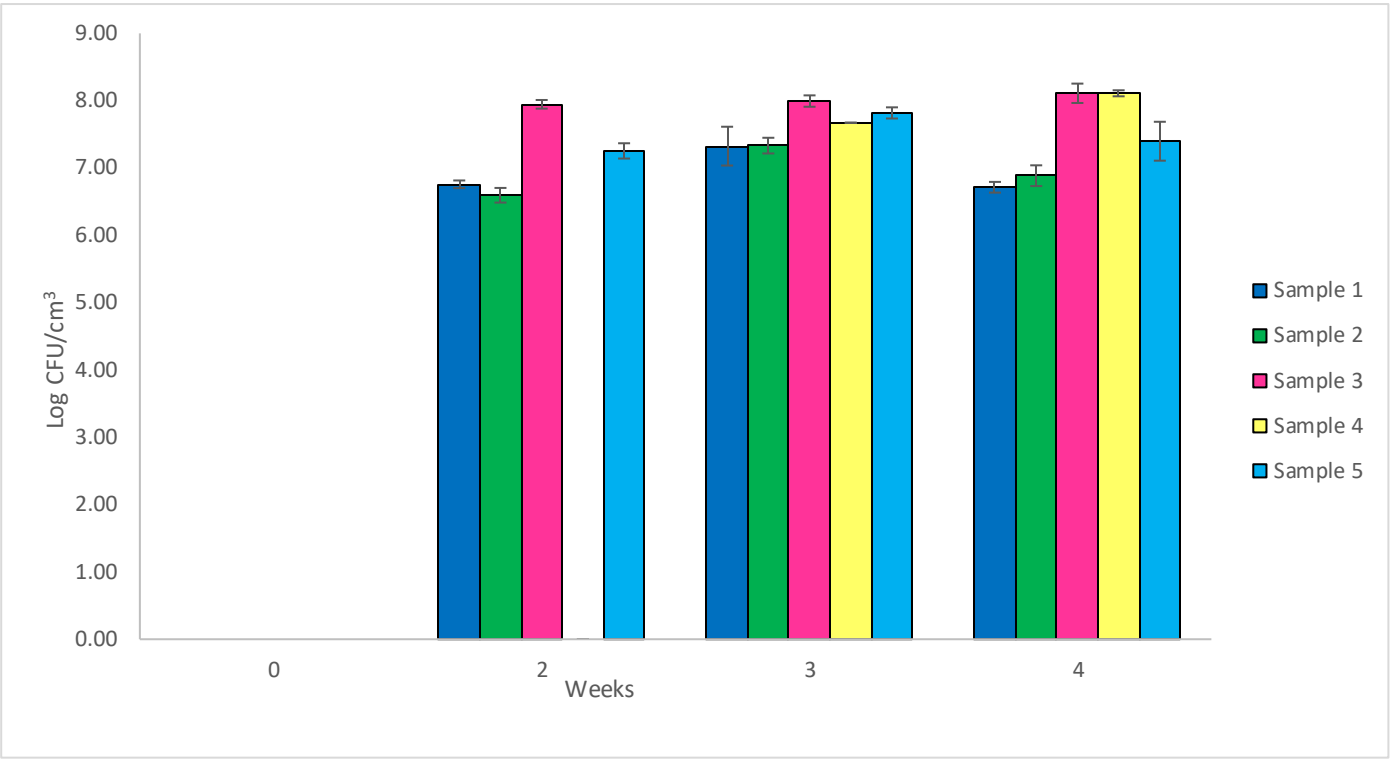

Figure 2 Bacterial abundance from kitchen sponges. The average weekly microbial content was calculated by scoring the number of CFU from each sponge. Each bar represents the average CFU counts between each of the participant's three samples. The error bars represent the standard deviation from three replicate samples. The P-Value for all sample is >0.1, indicating no significant difference from week to week.

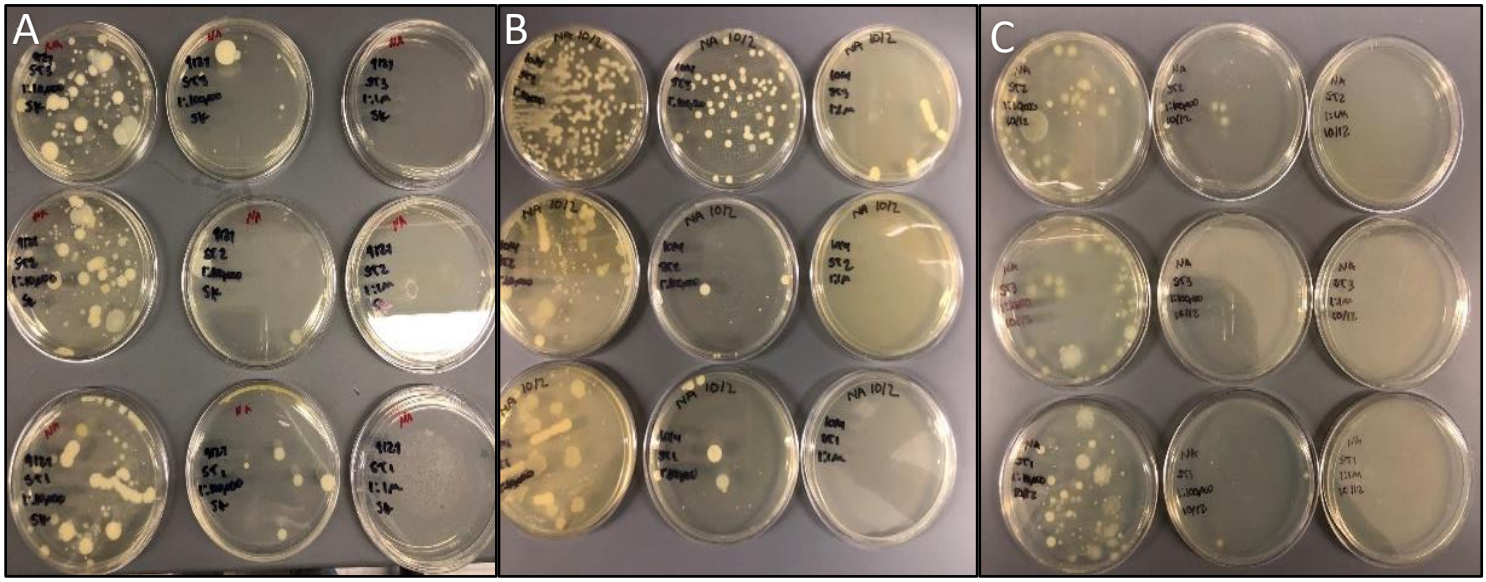

Figure 3 One representative example of weekly bacteria on standard nutrient rich agar plates. This is a representative example of the bacterial growth from a dilution series of one sponge on nutrient agar plates. Three once centimeters samples were taken from a single sponge and mixed with NB. Liquid recovered was subjected to 10-fold dilution series and the dilutions were plated onto a NA plate. This was conducted in triplicate, as indicated by the three rows. Each column displays a different dilution series of 1:10,000, 1:100,000 and 1:1,000,000 from left to right. Bacterial growth after A) two weeks, B) three weeks, and C) four weeks.

Table I. Comparison of microbial contamination in this study of kitchen sponges to published research

	Average bacterial number	**Sample Number (sponge)**	**Notes and Observations**
Cardinale et al.,2017	------	14	*Gammaproteobacteria* was seen to be the most prevalent class at 51.4%
Quinlan et al., 2017	41,686 CFU/cm^3	100	Sponges were seen as the most bacterial containing item
Wolde et al., 2016	10,000,000,000 CFU/cm^3	201	98.7% of their sponges had a bacterial count greater than 10,000,000,000 CFU/cm^3. *The* bacteria most prevalent was the genus *Pseudomonas* followed by the class *Bacillus*
Rossi et al., 2012	1,258,925,411 CFU/sponge	40	76.25% presented with CFU, ranging from 31,622- 7,943,282,347 CFU/sponge
Current Study	50,118,723 CFU/cm^3	5	The average count in sample sponges after 2-4 weeks of daily use.

Sanitation Techniques Applied to Used Sponges

The physical and chemical environment of a sponge has been shown to support bacterial growth, however, sponges are routinely cleaned and regularly discarded. There is not common consensus about the best way to clean a sponge. To address this, this study investigated the impact of cleaning on bacterial number after used sponges were cleaned. Different sanitation techniques were used to determine what bacteria treatment was the most effective (Table II). This was conducted in duplicate as sponge pieces from each of the five four week-used sponges were subjected to one of the five different cleaning methods. After each sponge piece was treated, it was placed in 10mL of nutrient broth, diluted and plated on NA. The untreated sponge pieces from each of the participant's sponge (negative control) received no disinfecting treatment. To score bacterial content, a standard dilution series following by CFU count showed an average count of 7.5 log CFU/cm^3 between each of the five untreated samples.

The results suggested that the use of 10% household bleach and 70% ethanol were the most effective methods to kill bacteria with both only having a total count of 4.6 log CFU/cm^3. Microwave treatment significantly reduced bacterial content in sponge pieces having an average bacterial count of 4.7 log CFU/cm^3. Dishwashing treatment and use of 55^{o}C soapy water showed the least decrease in bacterial contamination with total counts of 6.7 and 7.4 log CFU/cm^3, respectively. The figures below show the raw data CFU count and the data displayed as log CFU/cm^3 (Figure 4A and 4B). A representative image of the bacterial growth on NA plates after different sanitation treatments is displayed in Figure 5.

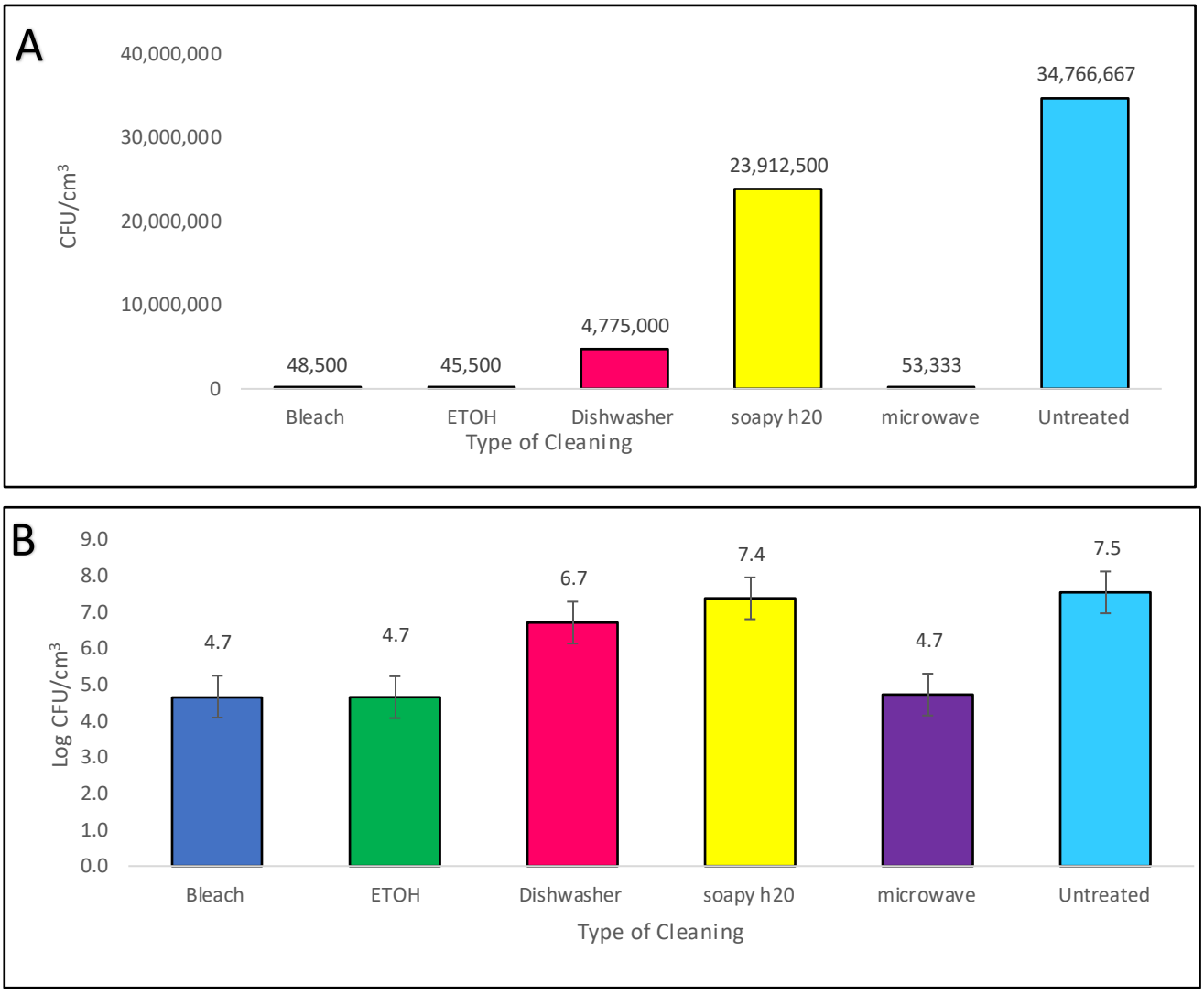

Figure 4 Microbial CFU count after cleaning methods of four-week-old sponges. Sponges were collected after four weeks of use and scored for CFU count after being treated with either 10 % bleach for one minute, 70% ethanol for one minute, dishwasher, soapy water at 55° F for one minute, microwave for one minute on high or left untreated in (A) CFU raw counts and (B) CFU log scale. The average amount of bacterial growth of the week four-used sponges is displayed as the untreated sample. The error bars represent standard deviation between each of the participant's sponge samples. The P-value from the unpaired one tale T-test was <0.05 for all cleaning methods against the control except for the use of soapy water at 55° C (P= 0.06), which suggests that the difference is significant and not caused by chance.

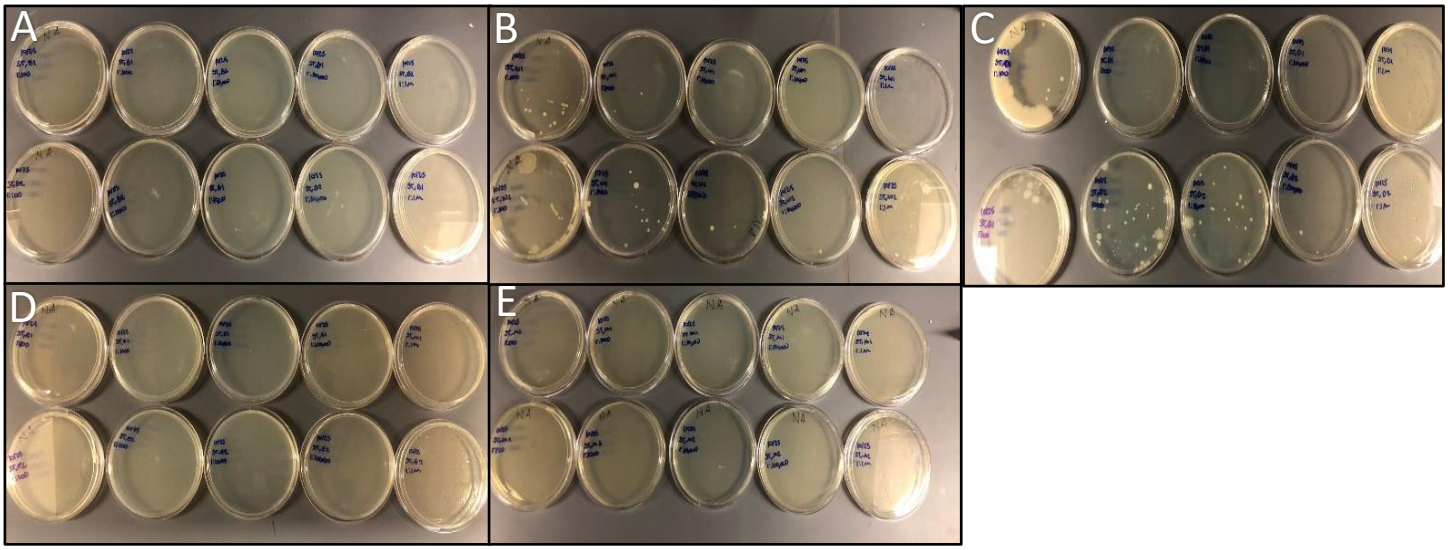

Figure 5 Representative image of bacterial growth (dilution series and CFU count) of one sponge after cleaning techniques. Panels show growth after 10% bleach (A), 55º F soapy water (B), dishwasher (C), 70% ethanol (D), microwave (E) on four week-used sponge pieces. Following sanitation, these pieces were placed in NB, vortexed for one minute, and diluted. NA plates were inoculated using the spread plate technique from the different dilution. The columns for each panel show the different dilution factor that was plated using the spread plate technique. Starting from left to right the dilution factor is, 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000. Each row shows a dilution series conducted in duplicate.

Table II. Comparison of the effects of bacterial reduction from different sanitation techniques between past studies. NT= not treated.

	Current Study	Sharma et al., 2009	Tate, 2006	Rossi et al., 2012	Ikawa and Rossen 2012	Cardinale et al., 2017
Dishwasher	86.0%	100.0%	57.3%	NT	100.0	NT
Microwave	99.8%	100.0%	29.7%	NT	100.0	0
Hot h20	31.2%	7.4%	NT	NT	NT	0
Bleach	99.9%	1%	NT	96%	NT	NT
Boiled	NT	NT	47.2%	100.0%	NT	NT
lemon	NT	20.6%	NT	NT	NT	NT
Vinegar	NT	NT	NT	NT	1.0	NT
Washing machine	NT	NT	0.4	NT	100.0%	NT
Ethanol	99.9%	NT	NT	NT	NT	NT

Transfer of Bacteria from Sponge to Counter Surface

From the previous experiments it was clear that there was an abundance of bacteria residing in the kitchen sponges, but whether the bacteria within the sponge has the capability to move to a new surface including eating surfaces and dishes is not entirely clear. To address this, a four week-used sponge was used to investigate the transfer of bacteria from sponge to surface (Figure 6). The contaminated sponge (7.3 log CFU/cm^3) was soaked in dH_2O, squeezed to release excess H_2O, and then scrubbed against an unsanitized marked off 10x10cm lab bench for one minute. The area was swabbed, and the swab was diluted in NB before being plated on NA. The bacterial abundance of that scrubbed area was found to have a count of 4.6 log CFU/cm^3. This was compared to the bacterial presence on a non-scrubbed lab bench area 2.1 log CFU/cm^2 and the bacterial abundance on an area that was scrubbed with a new sponge 2.2 log CFU/cm^3. The data shows a 5.12% bacterial transfer from sponge to surface after factoring the bacterial contamination already present on the lab bench.

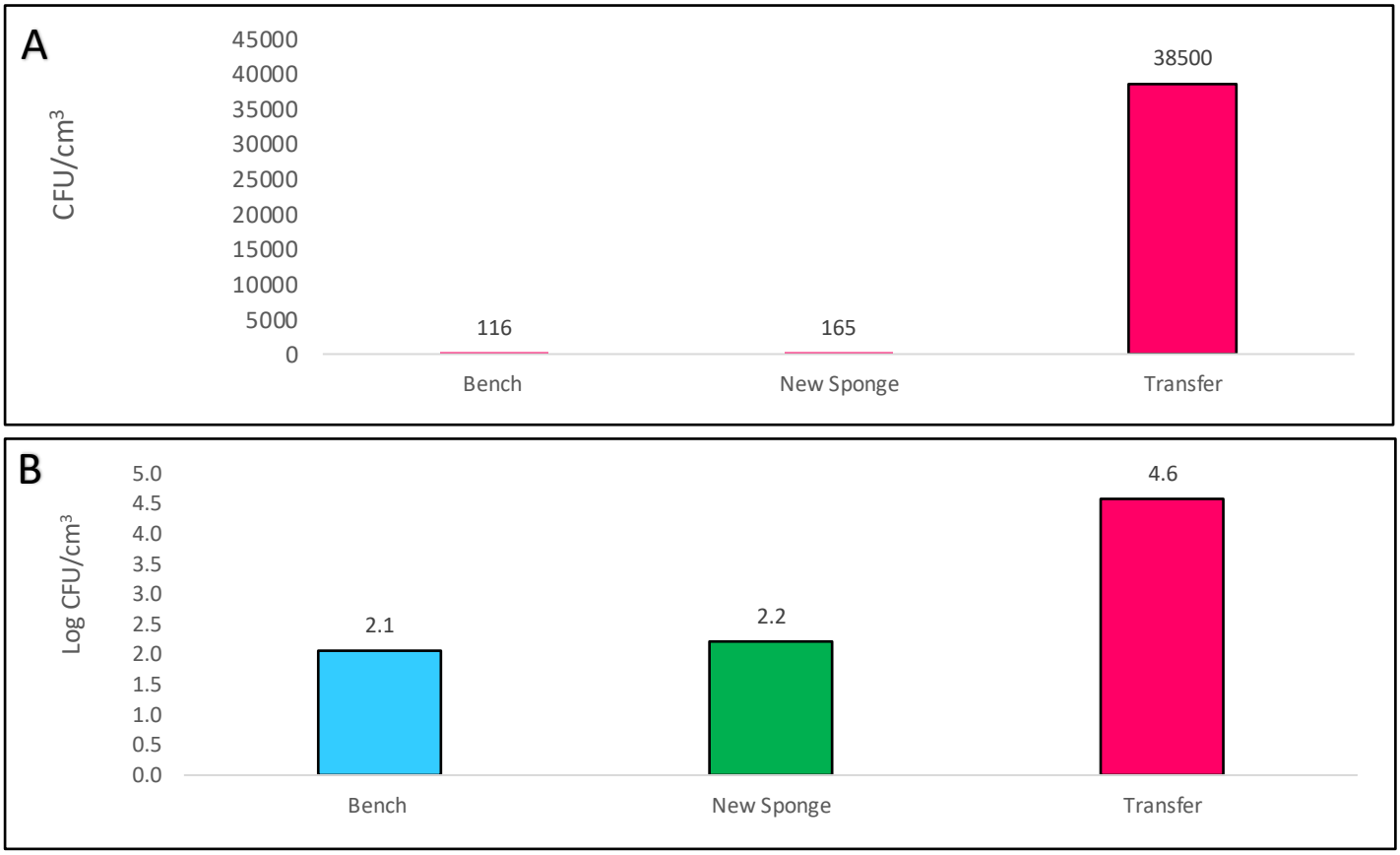

Figure 6 Analysis of bacterial transfer from surface to sponge. The transfer of bacteria from sponge to surface (typical non-sanitized lab bench) was calculated using a used four-week-old sponge containing a bacterial contamination of 18,600,000 CFU/cm^3 (7.3log CFU/cm^3). One-third of the used sponge was rubbed in a 10x10cm^2 marked off area. A new sponge was rubbed in a second area, and the third area was left untouched (Bench). The three areas were swabbed, mixed in NB and underwent a 10-fold dilution. The dilutions were plated on NA, and the bacterial growth was quantified after 48 hours at 37° C. The "Bench" bar indicates the bacterial abundance solely on the surface used in this experiment. The "New Sponge" bar shows the transfer of bacteria from a brand-new sponge to a surface and the third bar labeled "Transfer" displays the transfer of bacteria from a four-week used sponge to surface. The data is shown in total bacterial number (A) and log counts (B).

Discussion

Bacterial Abundance over a Four-Week Period.

Kitchen sponges are infected with a high number of bacterial microorganisms. It was predicted that the bacteria number would gradually increase from week to week, however bacterial abundance remained very high within two weeks and stayed relatively constant between week two and four. There was only an average 0.4% log increase of bacterial abundance from week two to week four used sponges. Although results were not available from the first week of use, by the second week, bacterial abundance (> 6.6 log CFU/ cm^3) was strikingly high for all samples in these used kitchen sponges. Using plate count techniques on nutrient agar, it was found that these sponge samples had a bacterial number ranging from 6.6 log CFU/cm^3 - 8.1 log CFU/cm^3 (3,066,667 CFU/cm^3 - 115,000,000 CFU/cm^3), with an average of 7.7 log CFU/cm^3 count in sample sponges after 2-4 weeks of daily use.

Similarly, high bacterial counts were found in past studies. Wolde *et al* observed that 98.7% of the 207 kitchen sponges evaluated had a mesophilic bacterial count greater than 10 log CFU/cm^3 *(Wolde et al., 2016)*. Rossi *et al* also found bacterial counts ranging from 4.1 to 10 log CFU/sponge with an average of 6.8 log/sponge *(Rossi et al., 2013)*. Since their study quantified the amount of bacteria present on the whole sponge not one square centimeter, it would be predicted that these counts would be far less than this current study and others which only looked at a small portion of the sponge. However, the lack of bacterial abundance in Rossi *et al* study may be because these sponges were used in industrial kitchens by food handlers trained in Good Manufacturing Practices with a "professional nutritionists controlling the food preparation and sanitizing procedure" (*Rossi et al, 2013)*. In addition, the study did not note the amount of time that kitchen sponges were used. Although the exact duration was not specified, the text states

"sampled sponges were used for at least one day", unlike this study which tested sponges that were used for four weeks *(Rossi et al., 2013)*.

These high level of bacterial contamination do not take into account the plate count paradox which states that the vast majority of microorganisms are unculturable. This means that the quantified bacterial load in this study does not include those bacterial cells that cannot be grown on nutrient agar and suggests an even greater contamination of these kitchen sponges. However, looking at the microbial growth on nutrient agar is efficient since it roughly demonstrates the number of heterotrophic bacteria that would grow in or on human beings. Alternative methods to visualize and calculate the high mesophilic load in kitchen sponges would be to use fluorescent in situ hybridization (FISH) as in Cardinale *et al's* study (2017) and/or calculate the average optical density of the sponge using spectrophotometer as in Tate's study (2006). Both techniques require the microorganisms to be grown/visualized in order to quantified. Another way to identify the abundance and species found in kitchen sponge's microbiome would be to use multiplex pyrosequencing. Using the 16s rRNA gene (present in all bacteria), the DNA for all the bacteria could be isolated within the sponge and then sent out for sequencing from performing PCA by amplifying the 16s rRNA gene. The DNA sequence that returns could be analyzed using bioinformatics (Blast) to determine all the bacteria in the sample that makes up the microbiome of the sponge.

Cleaning Methods

This study demonstrated that the use of chemical treatments (70% ethanol and 10% bleach) on used kitchen sponges had the largest impact with an average bacterial reduction of 99.9% (Table II). A microwaving treatment of one minute also displayed a reduction of bacteria number by 99.8%. Our results disagreed with a previous study that also investigated the effect of the dishwasher, washing machine, and the microwave as agents of sanitation *(Tate,2006)*. The research of this study found that microwave treatment on kitchen sponges for either 30 seconds or 60 seconds appeared to "have had the same effect on the sponges as if they had not been treated at all" (*Tate, 2006)*. The researcher's belief was that the thick, porous nature of the sponge provided the microbes protection against the heat.

Sharma *et al* found that microwave treatment of contaminated kitchen sponges was an effective method of killing bacteria, however the researchers did not see a significant log reduction in sponges that were treated with 10% bleach as this study did *(Sharma et al., 2009)*. Their data showed that kitchen sponges which were soaked in 10% bleach for three minutes were seen to have an average count of 6.1 log CFU/sponge compared to untreated sponges receiving no disinfecting treatment that contained 7.5 log CFU/sponge (Table II). The researchers suggested that the sodium hypochlorite found in bleach solutions was not able to penetrate a possible biofilm formed by the bacteria in the sponge *(Sharma et al., 2009)*. Both studies followed a similar protocol, however Sharma *et al.,* study treated their sponge pieces in the bleach for a longer period of time (3 min vs 1 min), which should have even decreased the bacterial load even more. Another study was conducted by Rossi *et al.,* which agreed with our current study showing a 99.9% bacterial reduction when kitchen sponges were placed in 0.02% sodium hypochlorite solution for ten minutes. These sponges were collected from Brazilian

restaurants and contained bacteria counts ranging from 3.4 to 10.4 log/sponge before the disinfection process *(Rossi et al., 2012)*.

Our study hypothesized that the dishwasher would be the most effective method, as the bacteria would die from the high temperature water and chemical treatment from the liquid dishwashing detergent. In addition, the water jets inside would disrupt attachment of bacteria to the sponge surface. Two previous reports found that the dishwasher was found to be the most effective method with an average of 100% mean percent reduction of bacteria between used kitchen sponges for each study (Table II) *(Sharma et al., 2006* and *Ikawa and Rossen, 2012)*.

In this current study, sponges placed in the dishwasher resulted in a bacterial reduction of 86%, failing to support the hypothesis, as other methods were seen to be more effective. The subsequent handling of the sponges that were subjected to the dishwasher were treated differently than the other cleaning techniques after sanitation which may be a factor that led to a lower bacterial reduction. For example, unlike the other sanitized sponge pieces, these sponge pieces were transferred to nutrient broth 1-5 hours after the dishwashing cycle ended to stimulate how sponges would be used in a household. The prolonged transfer period may have given the remaining bacteria present in the sponge after treatment time to recolonize which would increase the overall bacterial abundance found in these sponges. To minimize this variable in the experiment, all sponge pieces should have been treated equally before and after sanitation to receive the most accurate results. After undergoing a dishwashing cycle, sponge pieces should have been placed in nutrient broth, diluted and plated immediately, as the other samples were.

The use of water at 55^{o} C mixed with soap did not have a significant effect on the bacterial load, as these sponges contained comparable levels of bacteria to the untreated sponges. This is different than a previous study which saw an overall decrease in bacterial abundance with

the use of "special cleaning procedures" (*Cardinale et al., 2017)*. These researchers found a decrease in bacterial load when two-week and eight-week old kitchen sponges were either sanitized with the microwave or hot soapy water (45° C). Their study did not identify which type of cleaning technique was used on these sponges, which is problematic when attempting to compare this current research data with their data. Our current data showed a significant difference in bacterial reduction between treatments of hot soapy water and microwave (1% vs 99.8% reduction) (Table II).

The use of hot, soapy water to disinfect kitchen sponges is not recommended based on the results. Sponges should be either treated chemically, microwaved, or even run through a dishwasher to ensure the maximum amount of disinfection. Clorox even has a *Tips on How to Clean a Sponge* website page where it provides instructions from "experts" on how to properly sanitize sponges using their bleach products *(The Clorox Company, 2015)*. Their recommendation is to mix a half a cup of their Clorox Regular Bleach$_2$ product with one gallon of water and to let the sponge soak for five minutes following rinsing and drying. The Environmental Protection Agency direction for use of Clorox Bleach to sanitize sponges requires a higher concentration of bleach. Their directions include placing the used sponge in 3/4 cups of Clorox bleach to one gallon of water for at least one minute *(EPA, 2011)*. The USDA recommends using a microwave or a dishwasher to efficiently clean sponges, based off the research of Sharma *et al*, scientists at the Agricultural Research Service Food Technology and Safety Laboratory (ARS). The ARS which is the principal scientific research agency for the USDA found that microwaving sponges showed a 99.9999% reduction of bacteria while dishwashing showed an equally promising 99.9998% reduction of bacteria (Table II) *(Sharma et al., 2009)*. The efficacy of the microwave treatment was found to be similar in this study,

however the dishwasher treatment was not, again leading to the possibility of mishandling after sanitation. Although no FDA or EPA guidelines could be found on the benchmark of bacterial reduction in kitchen sponges to be deemed efficient, the sanitation standard for food contact surfaces is accepted at 99.999% (five log) reduction and the sanitation standard for non-food contact surfaces is at 99.9% (three log) reduction *(FDA, 2009)*. These guidelines can be used as a comparison for what is considered an adequate cleaning method for kitchen sponges based on the bacterial reduction number

Bacterial Transfer from Sponge to Surface

The third aim of this study was to investigate the transfer of microorganisms from sponge to surface. There have been multiple concerns that sponges not only provide a moist hospitable environment for microbes, but they also provide bacteria a means of transmission. The results from our study showed that that 5.12% of the germs in a four-week used sponge transferred to a hard-non-porous resin surface counter. This percent transferred was seen to be much lower than in previous studies which showed that 21-43% of bacteria present in a sponge can be transferred to different surfaces *(Rossi et al., 2013)*. This study artificially contaminated sponges with 10mL of bacterial suspension containing either *Staphylococcus aureus, Staphylococcus enteritidis, and Campylobacter jejuni*. The contaminated sponges were then wiped on a 50x80 cm^2 stainless steel surface using the contact plate method *(Kusumaningrum et al., 2003)*. The researchers found the transmission was not dependent on the type of microorganism, as this process was repeated with sponges infected with the different types of bacteria. Following contamination of the surfaces from the sponge, either a piece of roasted chicken fillet or a slice of cucumber was placed on the surface. There appeared to be a 50-100% transfer rate from the

kitchen surface to the chicken, and a 20-100% transfer rate from the surface to the cucumber suggesting that cleaning with contaminated sponges increases the risk to exposure to pathogens to food items *(Kusumaningrum et al., 2003)*.

We observed a more modest bacterial transfer in this study which may be due to a colonized bacterial biofilm in the sponge, that did not allow the liberation of the microorganisms when rubbed. A biofilm is a film of bacteria that adheres to the surface, in this case the sponge. As previously mentioned, Cardinale *et al* found a dense biofilm-like structure on the surface the used kitchen sponges they collected from German households using 3D visualizations (*Cardinale et al., 2017)*. The previous two studies artificially inoculated their sponges and used them right away, potentially not allowing enough time for the bacteria to form a biofilm. An experimental study found that it takes two to four days for planktonic bacteria such as *Staphylococci, Pseudomonas and E.coli* to form a full biofilm (*Phillips et al., 2010)*. Another reason for the reduced transfer is that in this study was that only about a third of the sponge was rubbed against the surface. Although not specified, in past studies the whole sponge could have come into contact with the surface, liberating more microbes from the sponge to the surface.

Future Directions

This study showed a vast amount of contamination in a kitchen sponge by the second week, so it would be interesting to see how soon bacterial growth starts in kitchen sponges and how rapidly it occurs until it reaches its plateau in number as we saw by week two. To conduct this research, the bacterial enumeration on kitchen sponges should be investigated within the first two weeks of use. Every day for two weeks a piece of the used sponge could be cultured in a similar manner to this study to visualize exactly when the bacteria starts to colonize the sponge.

Another direction to further this research would be to analyze the effects of bacterial recolonization after the sponges have been treated with different cleaning methods. This could be done with multipyrosequencing to get at the true number and diversity of microbes living in a kitchen sponge. The diversity of microbes found within the sponge's microbiome could be compared before and after treatment, to see if there was a certain type of bacteria that appeared to be more resistant than others. As mentioned before, Cardinale *et al.*, study showed an increase in bacterial number of two different families present in the sponge after the sponges were treated *(Cardinale et al., 2017)*. We could also see if different cleaning techniques targeted different types of bacteria using multipyrosequencing.

Recently there has been a surge for the demand of eco-friendly green cleaners and organic products as people are being more consciousness about the planet and the products they are using, consuming and wearing. Future studies should be aimed at testing these greener methods to determine their ability to disinfect on kitchen sponges. Vinegar is seen as a "green cleaner" and has been used to disinfect and eliminate odors, however is not considered a true disinfectant according to the EPA due to its lack of testing and certification *(Dickey, 2013)*. A

previous study showed that the use of vinegar to sanitize two to ten day used kitchen sponges provided a 98.6% bacterial reduction *(Ikawa & Rossi, 1999.).*

Concluding Remarks

This study provides us a better understanding of the immense amount of microbial number present in kitchen sponges. It also gave us provides empirical evidence about which cleaning methods are efficient in sanitizing sponges. This study illustrated that kitchen sponges can act as a vector leading to cross-contamination moving bacteria from sponge to surface. Our suggestions include disinfecting kitchen sponges more often since astonishing bacterial counts were seen as early as two weeks. Cleaning sponges in the microwave or with 10% bleach or 70% alcohol may reduce foodborne illness via cross-contamination in the home. Kitchen sponges are commonly found in household kitchens and were seen to contain high bacterial counts by the second week of use. Our 12-week study shows that it is necessary to disinfect kitchen sponges to reduce cross-contamination in the home, especially if living with someone who is immunocompromised and more susceptible to fall ill from foodborne pathogens.

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