JOURNAL OF
EMERGING INVESTIGATORS

Article

Effects of Cleaning Agents on Bacterial Growth in Refrigerator Surfaces

Chrestiane Tekola[1], Choah Kim[2], Jeffrey J. Li[3]
[1]Thomas Jefferson High School for Science and Technology, Alexandria, Virginia
[2]Harvard University, Cambridge, Massachusetts
[3]Johns Hopkins University, Baltimore, Maryland

SUMMARY

Foodborne illness is a critical public health issue, affecting millions of people worldwide. To combat this, preventative measures at both the individual and industrial levels have been implemented successfully, such as refrigeration and the prevention of cross-contamination. To demonstrate the importance of individual preventative measures, we tested different cleaning methods and how they might reduce the bacterial population present on surfaces of a home refrigerator. By measuring bacterial growth on LB agar plated with samples from a household refrigerator shelf before and after cleaning, we observed that cleaning shelves with water proved ineffective when compared to other agents such as dish soap and Lysol, which successfully reduced the number of surface bacteria. This work demonstrated the importance of cleaning agents in proper food safety measures.

INTRODUCTION

Foodborne illness is a critical public health issue, with 48 million Americans affected every year (1). In response, federal institutions such as the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) have been tasked with monitoring the spread of foodborne disease (2,3). Strict sanitation and food handling measures were enacted to prevent any outbreaks of foodborne illness. Examples of such practices include using clean water in different parts of crop production, sanitary storage of produce, and individual hygiene practices such as washing hands prior to handling foods (4). However, foodborne pathogen outbreaks may leave devastating impacts on various communities (5). In 1993, an *Escherichia coli* epidemic in the United States left 4 dead, and 178 victims were left permanently injured, highlighting the importance of public health measures to prevent foodborne illness (6).

Over 250 diseases are caused by consuming foods contaminated with harmful microorganisms (7). Although certain strains of *E. coli* are associated with foodborne illness, not all *E. coli* strains cause illness – most are harmless and can exist symbiotically in microbiota in the human body, such as in the gut (8). Composed of many species of microbes, the gut microbiota dwell in the digestive tract and aid in the digestive process (9). Pathogens, such as pathogenic *E. coli*, may disrupt the gut microbiota in a variety of ways, one of which is the creation of toxins that harm intestinal tissue during their reproduction (10). These toxins help the pathogen gain additional access to infiltrate the gut tissue (8-10). Toxins may assault other bacteria in their proximity, causing cell lysis or activating the immune system. As a result, pathogenic microbes can outcompete local tissues or symbiotic bacteria for nutrients, leading to pathogenic virulence, where virulent bacteria multiply and thrive at the host's expense (11). Additionally, pathogens may have varying strategies that aid in withstanding colonization resistance, such as altering surface proteins or hiding within cells in the body (11-12). Thus, when a pathogen colonizes the intestinal microbiota, it may result in chronic disease (13).

Far before the development of "germ theory" – the theory that infections and certain diseases are caused by the invasion of microorganisms — humans relied on several methods to prevent foodborne illness, such as preserving meat through drying (or desiccation) by using salt and sunlight (14). Reducing the available amount of intracellular water restricts bacterial activity, leading to decreased cellular hydration and enzyme activity, thus lowering the microbial growth rate (15). While it does not eliminate microorganisms, desiccation decreases the risk of significant microbial contamination that can otherwise cause disease to spread. However, resistant microbes may persist even after the process of desiccation (16). Aside from desiccation, modern technology has made refrigeration readily available. Low temperatures often limit or halt microbial growth and reproduction but may not always kill microorganisms (15,16). Nevertheless, cells are affected in a variety of ways by extremely low temperatures (16,17). When ice particles form, they harm the membrane by stopping chemical processes such as diffusion; in comparison to moderate temperatures, they slow the rate of growth (16,17).

In the modern era, there is an abundance of different cleaning solutions to cleanse microbial contamination on persons or surfaces (18) which can differ in their active components and mechanisms of action. For instance, ethanol, a commonly used solution in hand sanitizer, kills bacteria through protein denaturation, which is the process of unfolding proteins via chemical action, heat, or agitation leaving the protein unable to properly function (18). As a result, cellular processes are disrupted, leading to cell death. Additionally, ethanol can dissolve the cell membrane, leaving the cell unable to maintain a consistent internal environment and exposing its contents to outside molecules or stimuli (19).

Foodborne disease is a significant public health concern, affecting one out of every six Americans each year, but is mostly preventable (20). Individual preventative actions include hand washing, hygienic food storage, and cooking foods correctly (21). Hand washing promotes bacterial cell lysis and removal of the bacteria from the skin surface, which is critical for preventing cross-contamination (22). Cross-contamination is the physical movement or transfer of dangerous microbes

from one person, item, or location to another; it occurs just as frequently in storage areas as in the kitchen (23). For example, if ground beef fluids containing pathogenic microbes drips into yogurt, the yogurt becomes contaminated. Many harmful microorganisms, such as *Listeria monocytogenes,* may survive in refrigerators and grow between -1.5 and 45°C (24). Consumption of contaminated foods can subsequently lead to foodborne illness. Thus, basic food safety measures are critical in reducing these risks.

With foodborne disease being a major public health concern, we evaluated how modern sanitation practices influence bacterial growth. We examined bacterial samples from a refrigerator shelf before and after cleaning with different agents. We hypothesized that cleaning a surface with different agents would reduce the quantity of bacteria present, but these methods would differ in effectiveness. We investigated which readily available cleaning solutions were most efficient in suppressing bacterial development. We observed that cleaning with water did little to reduce the bacterial growth on both LB agar plates and in liquid cultures when compared to dish soap or Lysol disinfectant, which proved to be highly effective. These results highlight the importance of using additional cleaning agents beyond water to sterilize household surfaces.

RESULTS

To determine whether we could observe the initial bacterial population present in our sample, we swabbed a fridge shelf, mixed the samples with soy broth, and spread a dilution of each sample onto standard LB agar plates (**Figure 1**). We either swabbed the fridge shelf and applied it directly to the plate or plated a soy broth dilution (**Figures 2 and 3**). To confirm that our LB agar plates are capable of supporting bacterial growth, we used toilet water as a positive control. We performed the experiment steps without adding any fridge samples as a negative control.

We observed a decrease in bacterial growth on the LB agar plates for samples originating from the swab of the "water-cleaned" shelf compared to swabs of the uncleaned shelf. Interestingly, potential contamination could be seen around the perimeter of the plates (**Figure 2**). The "dirty" plate swabbed directly with the fridge sample showed irregular colonies that were difficult to measure (**Figure 2**). While there

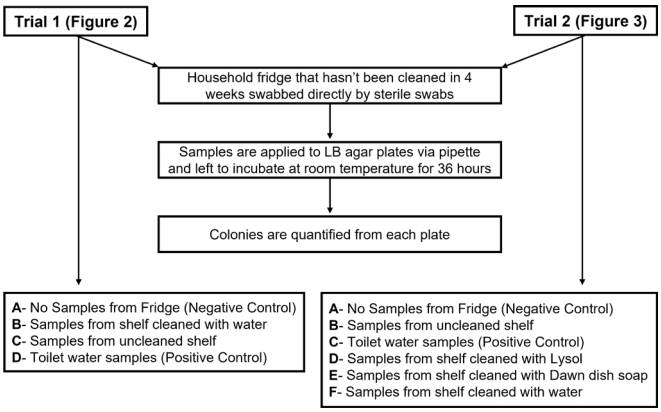

Figure 1: Experimental workflow of the two experiments. Letters denote the panels in Figures 2 and 3 showing the indicated plate.

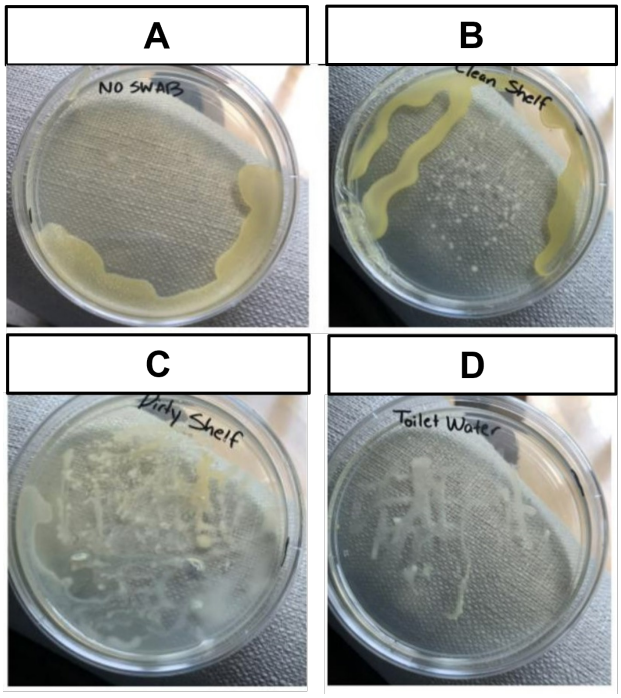

Figure 2: Colonies from fridge samples directly applied from the fridge to the agar plate. Photos were taken 48 hours after plates were left at room temperature. The plates contained samples from the following cleaning agents in the respective order: No samples from fridge (A), shelf cleaned with water (B), uncleaned shelf (C), and toilet water (D).

was no obvious contamination in the second trial after the experiment protocol was changed, the media on the plates showed little to no bacterial growth and instead displayed white 'watery' growths. As a result, for the next trial, a solution with a higher concentration and a smaller volume was plated (**Figure 3**). While the colonies could not be counted, the absence of growth on the clean shelf plate compared to the dirty shelf plate suggests that a basic cleaning of the shelf reduced the amount of bacteria present, as expected (**Figure 3**).

After demonstrating our experiment can resolve differences in surface bacteria populations as a result of cleaning, we ran a second experiment to discover which cleaning methods and products were bacteriostatic – i.e., capable of inhibiting the growth of bacteria but not necessarily eliminating them. While cleaning with water reduced the growth on the fridge shelf, we hypothesized that utilizing other cleaning agents such as Lysol and Dawn would result in less bacterial growth, as measured by growth after swabbing on LB agar plates.

For both experiments, we observed that the bacterial growth on the "dirty" plate was more than that on the positive control plate, and that there was no growth on the negative control plate after 36 hours of incubation (**Figures 2 and 3).** While the colonies were too numerous to count, there was a clear difference in bacterial growth between the dirty plates — plates containing samples from the fridge shelf before cleaning — and the clean plates — which contained samples from the shelf after cleaning with the various cleaning products (**Figure 3**). We concluded that cleaning the shelves with water was not as efficient as Lysol and Dawn dish soap at removing bacteria.

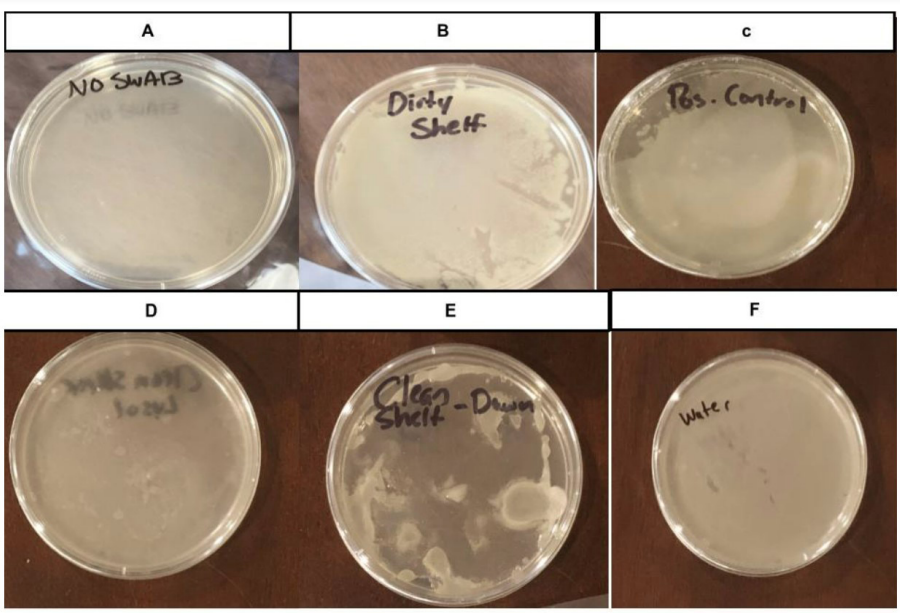

Figure 3: Bacterial growth from samples diluted in 1x TSB. Photos were taken after 53-58 hours of room temperature incubation after the plates had liquid culture spread on them. The plates contained samples from the following cleaning agents: No sample taken (A), uncleaned shelf (B), toilet water (C), household Lysol (D), household Dawn dish soap (E), and tap water (F).

DISCUSSION

We hypothesized that cleaning refrigerators would minimize the quantity of bacteria on their surfaces. Our initial experiment was not able to discern specific differences in cleaning solution used (**Figure 2**). This led to our second hypothesis that low concentrations of various cleaning products limit bacterial growth. Growth suppression occurred on some of the plates we examined, but not all of them (**Figure 3**). These included the plates containing samples from the shelf that had been cleaned with water (**Figure 2**). While the duplicates in the first trial exhibited a significant decrease in bacterial growth when compared to the colonies on the "dirty" plate, the plates cleaned with water in the second experiment showed no evidence of bacterial reduction (**Figure 3).** This decrease in growth may have been due to a protocol change, such as switching from direct application to subculturing, or as a result of contamination on the various plates (**Figure 3**).

Several of the findings were unexpected, such as the unusual growths in the dirty shelf LB agar cultures. In comparison to the colonies on the other plates, these growths were whiter and more fungal-looking in appearance. The contamination may have occurred due to an insufficiently sterilized environment. Another possibility is that the swabs holding the fridge sample were contaminated before being distributed onto the plates. There were also lawns of bacteria surrounding the bacteria, making it impossible to quantify their growth through colony counting (**Figure 2**). One possible explanation for the abnormal dirty shelf culture growth is that the shelf contained so much bacteria that swabbing directly from the shelf to the agar plate resulted in an excessive amount of bacterial growth. Another possibility is that the fridge shelf sample was contaminated with some other type of bacteria prior to the dilution in the agar plates. In the future, diluting by tenfold and being mindful of the surface area swabbed may combat this situation from occurring. An alternate technique for the second experiment with liquid cultures may be to wait 72 hours instead of 36 hours (**Figure 1)**, as it is likely that the cleaning products delayed bacterial growth rather than eliminating it altogether. This technique may result in a more precise difference between the samples at 1% concentration. If the experiment was repeated, the samples might be examined at lower concentrations to make the bacterial colonies more visible.

We found that the Dawn dish soap plate had fewer bacteria than the plate containing shelf samples cleaned with Lysol samples (**Figure 3**). We speculate that it is because soap can physically remove pathogens, but disinfectants such as Lysol do not necessarily do so. Another possibility is that the Lysol liquid cultures were diluted to the point where the bacteria could not grow adequately in 36 hours. In future experiments, a broader range of similar cleaning products might be used to determine if the different rate of growth was caused by the cleaning products' active components or by other factors.

While the results showed that cleaning the fridge shelf effectively decreased bacterial growth, we were unable to identify whether these growths were pathogenic or not. In the future, we may conduct a phenotypic test to recognize and identify different bacterial strains and species. We may also do DNA sequencing to examine and sequence the bacterial samples to identify their genetic information (24). This technique may then be used to identify bacterial strains and their harmfulness. This information would allow us to better understand how much of the bacterial populations in the fridge exhibit pathogenic characteristics, as well as how different cleaning agents suppress growth in different species of bacteria.

Previous research has concentrated on the biological and environmental factors that impact foodborne disease, as well as on methods to combat outbreaks from recognized as well as lesser-known foodborne pathogens (25). With the growing issues of food security and climate change in the modern age, food will be produced in altered natural environments

and will require novel methods of sustainable and improved production. Because the connections between these changes and the food system are complicated, there is uncertainty about the ensuing impacts. The potential for the emergence of new dangerous pathogens necessitates the development of preventative strategies to combat future outbreaks. Our research highlights the importance of developing targeted, preventative measures for these diseases by identifying which substances are most effective at hindering specific pathogenic species.

MATERIALS AND METHODS

Preparation of LB agar Plates

To make LB agar plates, 125 mL of LB agar was microwaved until it boiled. Then about 20 mL of LB agar was poured into each plate and left to cool for 8 hours. This process was repeated for the second experiment.

Experiment 1

To generate a "dirty shelf" sample from the refrigerator, one shelf was swabbed prior to cleaning; swabs were soaked in sterile water. The swabs were used to spread any bacteria evenly across LB plates and left to grow at room temperature for 36 hours.

After that, a part of the shelf was cleaned with water, swabbed, and then the sample was swabbed straight onto the LB plate. The plate was allowed to grow at room temperature for 36 hours.

Experiment 2

About 7 days later, for the second experiment, swabs were soaked in a 1:100 tryptic soy broth (TSB) solution. The same shelf was swabbed once before cleaning and then three times vertically after cleaning with water, Dawn dish soap, or Lysol. Swabs were then dipped into 0.5 mL of TSB and left to grow for 72 hours. Then, 2.5 mL of the liquid culture was evenly spread across the LB plate and left to grow for 48 hours at room temperature.

ACKNOWLEDGEMENTS

We would like to thank the JEI Mini-PhD program for providing the lab equipment for the experiments and introducing how to explore interests through research. We would also like to thank them for pairing students with a thoughtful and encouraging mentor who supported us through this process. Finally, we would like to thank Michael Mazzola for the helpful discussions regarding experiment design.

Received: October 13, 2021
Accepted: March 14, 2022
Published: March 11, 2023

REFERENCES

1. Centers for Disease Control and Prevention. (2018, November 5). *Estimates of foodborne illness in the United States*. Centers for Disease Control and Prevention. Accessed June 12, 2022.
2. Commissioner, Office of the. "When and Why Was FDA Formed?" *U.S. Food and Drug Administration*, FDA, www.fda.gov/about-fda/fda-basics/when-and-why-was-fda-formed.
3. "Our History - Our Story." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 4 Dec. 2018
4. "Timeline of Reported Cases." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 18 Dec. 2020
5. Murano, Elsa A, et al. "The Outbreak That Changed Meat and Poultry Inspection Systems Worldwide." *Animal Frontiers: the Review Magazine of Animal Agriculture*, Oxford University Press, 19 Sept. 2018 doi: 10.1093/af/vfy017
6. Commissioner, Office of the. "Food Safety at Home - Fact Sheet." *U.S. Food and Drug Administration*, FDA
7. "Foodborne Germs and Illnesses." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 18 Mar. 2020
8. Committee, The BC Cook Articulation. "Causes of Foodborne Illnesses." *Food Safety Sanitation and Personal Hygiene*, BCcampus, 15 May 2015
9. Ursell, Luke K, et al. "Defining the Human Microbiome." *Nutrition Reviews*, U.S. National Library of Medicine, Aug. 2012 doi: 10.1111/j.1753-4887.2012.00493.x
10. Rolhion, Nathalie Chassaing et al. "When Pathogenic Bacteria Meet the Intestinal Microbiota." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, The Royal Society, 5 Nov. 2016 DOI: 10.1098/rstb.2015.0504
11. Alberts, Bruce. "Cell Biology of Infection." *Molecular Biology of the Cell. 4th Edition.*, U.S. National Library of Medicine, 1 Jan. 1970
12. Zautner, A. E., et al. "More pathogenicity or just more pathogens?-on the interpretation problem of multiple pathogen detections with diagnostic multiplex assays." *Frontiers in Microbiology.* vol. 8, June 2017. doi:10.3389/fmicb.2017.01210
13. "Food Preservation through the Ages · the Natural Farmer." *The Natural Farmer*, thenaturalfarmer.org/article/food-preservation-through-the-ages/.
14. Stark, J M, and M K Firestone. "Mechanisms for Soil Moisture Effects on Activity of Nitrifying Bacteria." *Applied and Environmental Microbiology*, vol. 61, no. 1, 1995, pp. 218–221., doi:10.1128/aem.61.1.218-221.1995.
15. Wolaniuk, Monika. "How Does Soap Work?" *Hackensack Meridian Health*, www.hackensackmeridianhealth.org/en/HealthU/2020/08/11/how-does-soap-work#.Y11AQnbMLiA. Accessed 11 Aug. 2020
16. Clarke, Andrew, et al. "A Low Temperature Limit for Life on Earth." *PloS One*, 19 June 2013 doi: 10.1371/journal.pone.0066207
17. Nedwell, D.B. "Effect of Low Temperature on Microbial Growth: Lowered Affinity for Substrates Limits Growth at Low Temperature." *OUP Academic*, 1 Oct. 1999, doi 10.1111/j.1574-6941.1999.tb00639.x
18. "Environmental Cleaning Supplies and Equipment | Environmental Cleaning in RLS | HAI | CDC." *CDC*, 21 Apr. 2020,
19. Gutierrez-Mazzotti, Claudia. *UCSB Science Line*, UCSB Science Line, scienceline.ucsb.edu/getkey.php?key=5798. 4 Mar. 2017,
20. "Burden of Foodborne Illness: Findings." *Centers for Disease Control and Prevention*, Centers for Disease

Control and Prevention, 5 Nov. 2018,
21. "How to Prevent Food Poisoning | Food Safety | CDC." *Centers for Disease Control and Prevention*, 14 Aug. 2020
22. "Show Me the Science - Why Wash Your Hands? | Handwashing | CDC." *Centers for Disease Control and Prevention*
23. "4 Simple Steps to Keep Food Safe." *Eatright.Org - Academy of Nutrition and Dietetics*,
24. Center for Food Safety and Applied Nutrition. "*Listeria* (Listeriosis)." *U.S. Food and Drug Administration*, FDA
25. "Sequencing Genomes - Genomes - NCBI Bookshelf." *National Center for Biotechnology Information*
26. Smith BA, Fazil A. "How will climate change impact microbial foodborne disease in Canada?" *Can Commun Dis Rep.* 2019 Apr 4;45(4):108-113. doi: 10.14745/ccdr.v45i04a05.