Analyzing the Effects of Lysol Disinfectant Spray, Tap Water, and Vinegar on Bacterial and Fungal Growth of a Dorm Dining Table

Cindy Chang–chang.cindy04@gmail.com, Carolina Deng, Dia Mandric, Chloe McDonald

Abstract

This study investigates the effectiveness of three different typical household cleaning products on a university dorm kitchen table: Lysol disinfectant spray (LDS), tap water and white vinegar. The kitchen table was separated into nine quadrants and each treatment was applied to three quadrants for replication purposes. After treatment, swabs were taken from the quadrants and streaked onto agar plates, which were incubated for six days. Bacterial and fungal growth was observed over six days to assess percent coverage on the plates. A one-way ANOVA test revealed no statistically significant ($p > 0.05$) difference in growth rates between the cleaning products. These results suggest that the choice of cleaning product does not significantly impact the amount of bacterial and fungal growth on a kitchen table.

Introduction

Kitchens are considered to be the dirtiest place in one's household, even more so than bathrooms (NSF International, n.d). A 2019 study that sampled various tools, surfaces and devices regularly found in kitchens for fungal and bacterial contamination revealed that kitchen surfaces had the highest levels of contamination (Rahimi et al., 2019). When kitchen surfaces are not cleaned properly, the bacteria and fungi can result in foodborne illnesses like *Salmonella* and *Campylobacter* infections that affect the gastrointestinal system in people with weakened immune systems (Layton et al., 2006). However, when kitchen surfaces are cleaned properly, chemical treatments, like Lysol Disinfectant Spray (LDS), are more effective at reducing bacterial strains than natural treatments, like vinegar (Rutala et al., 2000).

There are limited studies addressing the effectiveness of cleaning products on both bacterial and fungal strains in relation to kitchen surface contamination. Our study aims to assess the effectiveness of natural (tap water and white vinegar) and chemical (LDS) treatments in reducing bacterial and fungal contamination on a university dorm kitchen table.

The objective of this study is to compare the average growth rate and average percent coverage of bacterial and fungal colonies after a six day observation period for three different treatments (tap water, vinegar, LDS) and a control (the dorm kitchen table prior to treatment) using a grid. This was achieved by swabbing the dorm kitchen table before and after treatments were applied, streaking the swabs onto agar plates, and placing them in an incubator at 37°C. Bacterial and fungal growth was then observed roughly every twenty-four hours over the course of six days. Based on previous research, LDS is expected to be the most effective treatment due to its active ingredient, quaternary ammonium, which disrupts the cell walls of microorganisms (Toxicology Regulatory Services, Inc., 2011). Therefore, agar plates treated with LDS and incubated at 37°C are expected to show the lowest average growth rate and the lowest average percent coverage at the end of the six-day observation period. Based on this literature, the null

hypothesis, H_0, is that cleaning products do not affect the growth of bacterial or fungal colonies and the alternative hypothesis, H_A, is that cleaning products do affect the growth of bacterial or fungal colonies.

Methods

To evaluate the effectiveness of the cleaners, a UBC dorm kitchen table shared by four roommates was intentionally left uncleaned for one week before treatment. First the table was divided into nine even quadrants using tape and labelled 1 through 9. Treatments were then assigned to the quadrants using a random number generator where each treatment (tap water, LDS, vinegar) had three randomly assigned quadrants. To assign the control quadrants, one of the three quadrants assigned to a treatment was randomly chosen. This process was repeated for each treatment to produce a total of three control quadrants.

Figure 1. UBC dorm kitchen table divided into its nine even quadrants. Treatments were assigned to quadrants via a random number generator. The numbers on the tape indicate the quadrant number and the arrows indicate whether it is to the left or right of the tape border.

The cotton swabs were lightly moistened in sterile water before collecting each sample. For the control, the swab was rolled across the respective quadrant in an X-shaped pattern (keeping this pattern consistent for each future quadrant) and placed back in its sterile packaging. For the treatment quadrants, 3 mL of the respective treatment was measured in a graduated cylinder and poured onto one of their assigned quadrants. It was wiped with a paper towel, and then left to sit for two minutes before rolling the swab on the surface.This process was repeated for each of the treatment groups, keeping the pouring and wiping method consistent. Observations were taken before the table was split into quadrants, as well as after so that it's possible to differentiate which quadrants may have had the dirtiest areas. The quadrants closer to the edges of the table (3, 5, 6, 9, 8) looked the dirtiest before treatment application. Observations

were also taken after all treatments were applied to the table to note that each quadrant had been fully cleaned and there were no clear dirty areas. Once all treatments had been applied, the samples were immediately taken to the lab to be transferred to agar plates. The agar plates were labelled with the treatment, quadrant number, and lab time before being streaked. To streak the plates the cotton swab was moved side to side from the top to the bottom of the plate. The sides of the agar plate were never touched with the sample. The plates were then sealed with parafilm and put in an incubator set to 37°C. The plates were left to grow for two days before being observed. On the first day of observation, the plates were taken out, observed, and photographed under a grid to note growth. There was already growth showing on many of the plates, although for most it was very few colonies. The exception to this was LDS #5 and Control #1 which had grown many colonies. The plates were checked every day after the first day of observation for a total of six days. Each day photographs were taken of the plates both with and without the grid underneath. During these observational periods it was important to note any changes in growth seen, any possible questions that might have formed from the data, or any other additional observations.

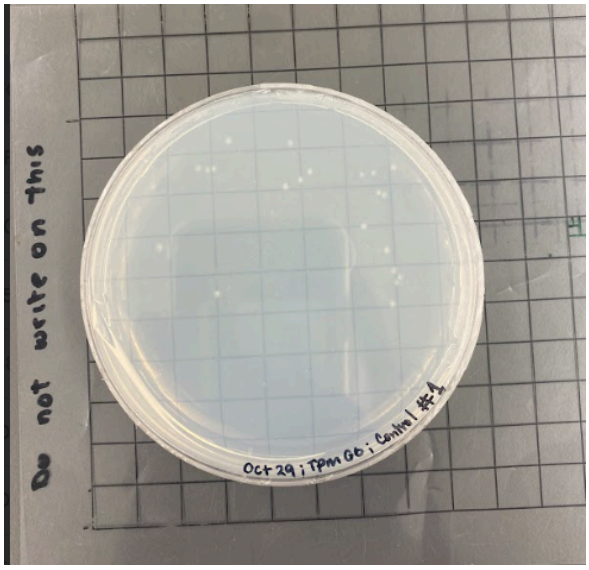

Figure 2. Agar plate being measured on grid to determine percent coverage. Agar plate shows zero percent coverage due to no squares being more than fifty percent filled.

To determine percent coverage, the bacteria or fungi had to cover at least fifty percent of a grid for it to be considered growth. The agar plates had black small dots, as well as larger translucent white colonies that were both used to determine percent coverage. The agar plates took up a total of fifty-five squares on the grid, and each square that had fifty percent or more coverage was counted to then find the total percent coverage. This was a randomised prospective study, and a one-way ANOVA test was used to determine the statistical significance of this study.

Results

The agar plates observed to contain bacterial and fungal growth had fuzzy transparent white spots that were usually clustered together, with the centre of these spots being an opaque yellow dot. Some of these plates also contained some round translucent spots shaped like a circle. Many plates were covered with a large amount of tiny black dots, but this was only noticed later into the experiment. The plates were also examined underneath a dissecting microscope to observe the morphology of colonies.

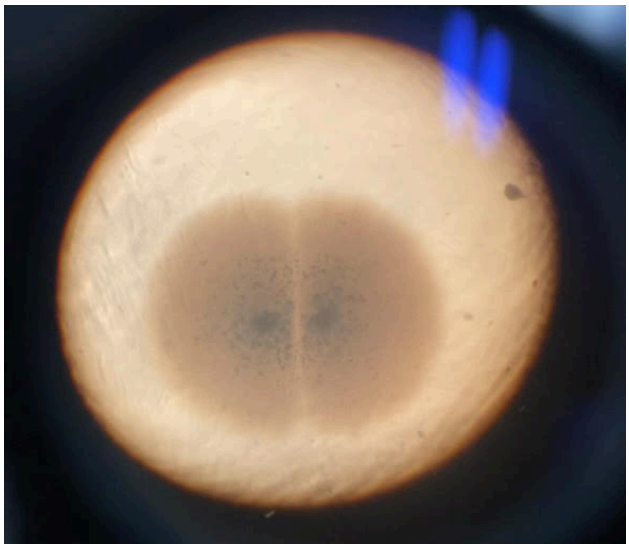

Figure 3. Image of a fungal colony viewed under a dissecting microscope on an agar plate. Taken on Day 2 of growth after treatment with LDS on quadrant 5.

Two group members calculated the percent coverage of bacterial and fungal growth on each plate (which included the tiny black dots and translucent white spots) while two group members counted the number of bacterial and fungal colonies on each plate (which only included the translucent white spots). The calculations were averaged like the following:

$$\frac{1st\ teammate's\ calculated\ \%\ coverage + 2nd\ teammate's\ calculated\ \%\ coverage}{2}, \quad (1)$$

and the same formula was applied to average the number of bacterial and fungal colonies counted between the two group members.

In this experiment, the treatment, or independent variable, was the cleaning products (separated into three categories: tap water, Lysol disinfectant spray, and vinegar) and the dependent variable was the percentage of bacterial or fungal colonies that covered the agar plate.

In order to run the one-way ANOVA test, the percent coverage of all replicates for a given treatment were plotted against one another for each day as a scatterplot(this was repeated for the control, tap water, Lysol disinfectant spray, and vinegar treatment). Since the growths were closer to an exponential trend than a linear one, a logarithmic transformation was applied to the percent coverage (y-values of the plot) for each scatter plot. From there, we used Google Spreadsheets to provide a regression line for each replicate and inputted the slopes of the regression line into the provided one-way ANOVA test calculator for the four treatments. After running the one-way ANOVA test with Analysis ToolPak on Microsoft Excel, the calculator

yielded a p-value of 0.169, indicating that the differences in bacterial and fungal percent coverage per day between each treatment was insignificant.

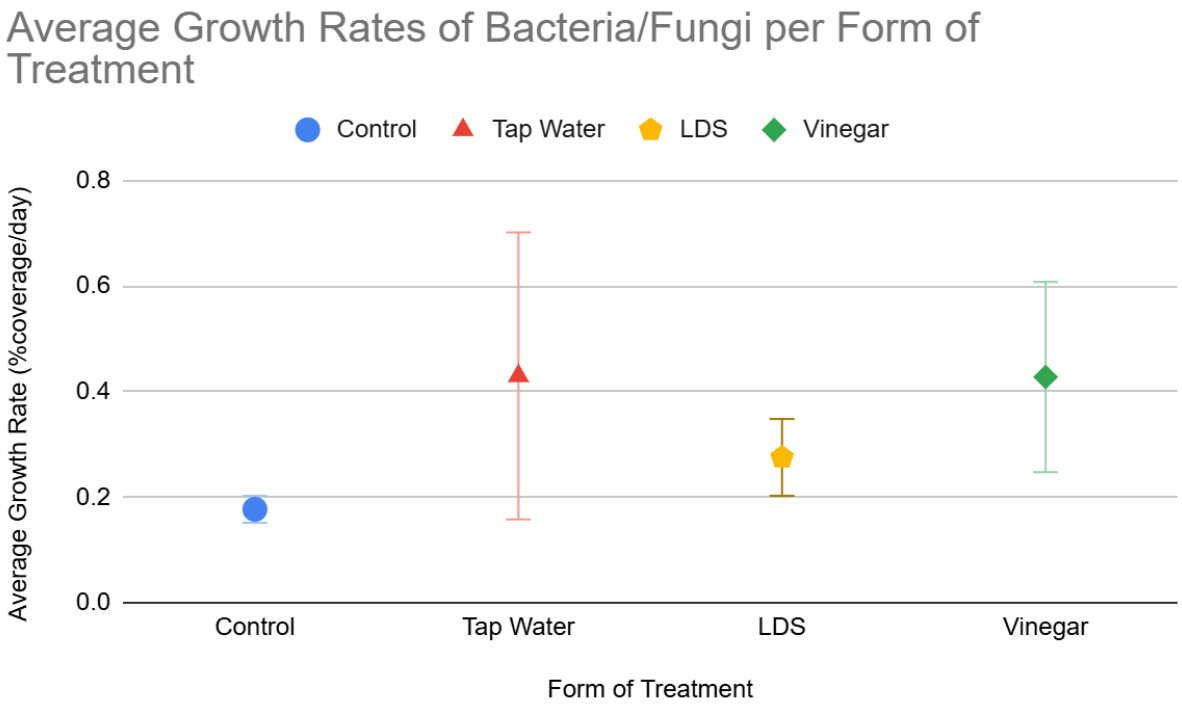

Figure 4. Average growth rates of bacteria and fungi per form of treatment (Control, Tap Water, LDS, Vinegar). Scatterplot made in Google Sheets depicting the average bacterial and fungal growth observed under each treatment: control (untreated), tap water, LDS, and vinegar. Data points represent the average growth rate, which was calculated by averaging the growth rates of the three replicates per treatment over the six day period of observation. Error bars represent the 95% confidence interval.

The control exhibited the lowest average growth rate of 0.177 ± 0.025, serving as a baseline for comparison to the three treatments. In comparison, tap water and vinegar both had similarly high average growth rates of 0.430 ± 0.272 and 0.428 ± 0.180 respectively, with vinegar being slightly higher With an average growth rate of 0.275 ± 0.073, LDS remained intermediate. All values are reported to a 95% confidence interval and in units of percent coverage per day.

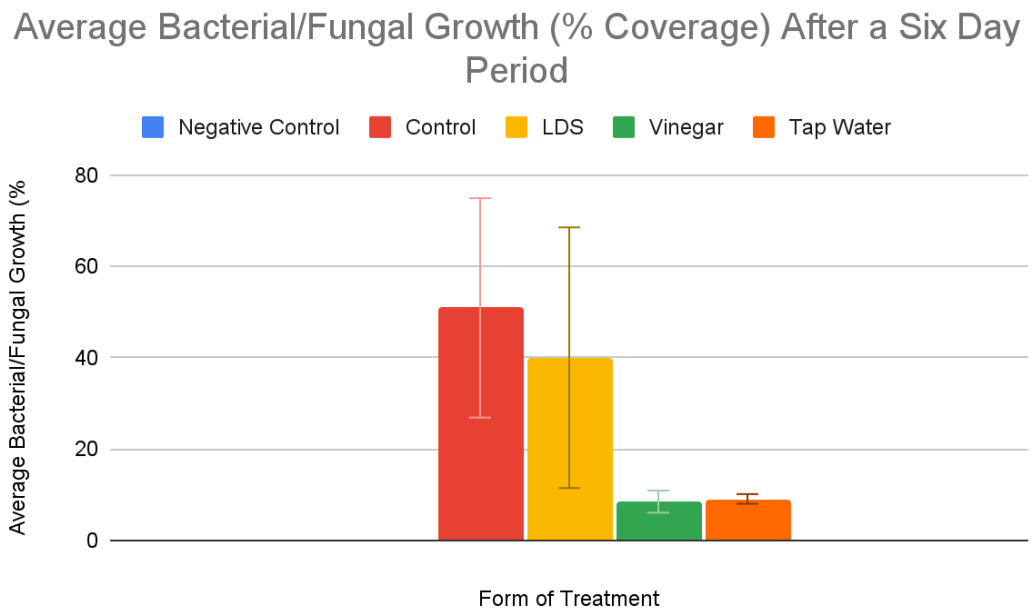

Figure 5. Bar graph depicting the average bacterial and fungal growth in terms of percent coverage on the agar plates after a six day period of incubation at 37°C. Error bars represent the 95% confidence interval. Percent coverage was calculated using a grid. The negative control bar is not present because all of its replicates had zero growth after the six day period.

The average percent coverage, reported to a 95% confidence interval, after the six-day period of observation for the control, LDS, tap water, and vinegar plates was roughly 50.909 ± 24.006%, 40 ± 28.536%, 9.091 ± 1.050%, and 8.485 ± 2.424% of the plate covered with growth respectively. On average, control and LDS had large percent coverages with wide 95% confidence intervals, while tap water and vinegar both had small percent coverages with narrow 95% confidence intervals.

Discussion

This experiment demonstrated the effectiveness of three different cleaning agents: tap water, LDS, and vinegar on reducing bacterial and fungal growth on a dorm kitchen table. It was hypothesised that if Lysol Disinfectant Spray is the most effective at disrupting bacterial and fungal cell walls, then it would result in the lowest growth rate and the lowest average percent coverage at the end of a six day period of incubation at 37°C. The reasoning behind this was that the active ingredient in Lysol Disinfectant Spray, quaternary ammonium, is more effective at killing microorganisms than natural products, as direct contact causes the destruction of cell walls (Toxicology Regulatory Services, Inc, 2011). A previous study that tested the effectiveness of LDS versus vinegar on reducing bacterial growth found that LDS eliminated bacterial growth more effectively (Rutala et al., 2000). However, this experiment yielded results that failed to

reject the null hypothesis, as the one-way ANOVA test for comparing the average bacterial and fungal growth rates between the three cleaning products indicated no significant difference.

Moreover, the figures also illustrate insignificant results, as Figure 5 displays overlapping 95% confidence intervals for the average bacterial and fungal growth (% coverage) on day six, indicating a possibility that there is no significant difference in average growth rates across the different treatments and the one-way ANOVA test done on Figure 4 did not display a significant difference in the average growth rate of bacteria and fungus. Even after including the 95% confidence intervals, an overlap of error bars supports the possibility of no significant difference. According to the data, the difference was most likely due to chance.

The insignificant results could be due to several reasons. Firstly, the LDS treatment could have been less effective due to the presence of organic material on the dorm kitchen table. Organic material such as saliva, dirt, and other debris present on the dorm kitchen table is one of the most common factors that can result in disinfectant failure (*Factors affecting disinfection,* 2023). It interferes with the disinfectant by chemically reacting with it, leaving less disinfectant to kill microorganisms. Organic material can also protect microorganisms from the disinfectant, making them harder to target. Secondly, different quadrants on the table had varying degrees of cleanliness, resulting in areas with different amounts of bacteria and fungus. For instance, one quadrant that was treated with LDS (number five) in particular was located at the centre of the table that had the most bacterial and fungal growth. Additionally, the distribution of bacterial and fungal growth on the plates may have influenced the results. When grids were placed beneath the plates to calculate percent coverage, growth that was more spread out was more likely to be undercounted compared to more clustered growth. For instance, if a plate contained a few colonies that were concentrated within a single square on the grid, all those colonies would contribute to the percent coverage of that plate. In contrast, a plate with numerous colonies spread across multiple squares would only count as having coverage in squares with more than fifty-percent growth, potentially leading to a lower overall percent coverage. Finally, there may not be a significant difference between the average growth rates due to errors made when counting percent coverage. On day five of observation, tiny black fungal spores became visible on multiple agar plates when they were placed over a white background. It is unclear whether this growth appeared before day five, as percent coverage was determined when the plates were on a dark grey background, where the black spores were less visible. The aforementioned errors would have resulted in inaccurate percent coverages and calculated growth rates, affecting the p-value generated by the one-way ANOVA test.

A limitation of this study includes the subjectivity of counting bacteria and fungal percent coverage on the agar plates and a small sample size. Each day, two different individuals counted the percent coverage for growth on all plates. However, differing interpretations of what constitutes more than fifty percent coverage in a grid square could introduce variability in the results. Furthermore, with only three replicates for each control and treatment group, the data is more susceptible to outliers and errors.

In the future, the results of this experiment can be improved by cleaning the surface before using the cleaning products to prevent organic material from affecting the effectiveness of them. Using a background colour that allows clear visibility of both light and dark bacterial and fungal growth is more optimal for calculating percent coverage. Lastly, having larger sample sizes for each control and treatment group can provide results that are less affected by errors and outliers.

Conclusion

This experiment aimed to analyze the effectiveness of different cleaning products in reducing the amount of bacterial and fungal growth on a dorm kitchen table. The results did not support the hypothesis and revealed no significant difference between the average growth rates across the different treatments based on a one-way ANOVA test. However, this may have been due to errors in experimentation. An area of future research could be evaluating the effectiveness of different cleaning agents on targeted microorganisms in a controlled laboratory setting.

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