

## Disinfectants and Salmonella: A Study Showing the Effectiveness of Disinfectants Against the Bacteria *Salmonella*

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### Abstract

This study was performed to determine the most effective disinfectant against *Salmonella* bacteria. Three disinfectant wipes and three disinfectant sprays were tested against the bacteria, Clorox, Lysol, and Safeway. The disinfectants were tested by placing *Salmonella* bacteria on Petri dishes. The disinfectants were then applied to the Petri dishes and dishes were incubated for both 24 and 48 hours. After each incubation time, the zone of inhibition was measured. An ANOVA and Tukey tests were done to analyze the data. Through these statistical analyses, Clorox spray was found to be the most effective disinfectant for 24 hour incubation ( $f = 43.88$ ; d.f. = 5;  $P < 0.0001$ ) and 48 hour incubation ( $f = 107.46$ ; d.f. = 5;  $P < 0.0001$ ).

### Introduction

Many household cleaners have been found to be effective against bacteria when used properly, but many times they are not used properly. Household kitchens have been tested and found to contain more fecal bacteria than household bathrooms, since antibacterial cleaners are used more often in household bathrooms (Aromatic News, 1995). Aromatic News found that fecal bacteria, such as *Escherichia coli* coliform bacteria, were not found in the bathroom as expected, but in the kitchen, on sponges and countertops. Other types of bacteria found in kitchens are *Salmonella* and *Campylobacter*, which originate from raw meat (Mattick, et al, 2003). When these bacteria are not disinfected properly, they can lead to contaminated food and food borne illness. Contaminated food can occur, for example, when a knife that touched raw chicken, comes in contact with cooked ready to eat chicken. The bacteria spread from the knife to the cooked chicken, which will be consumed. This may cause a food borne illness. This food borne illness affects the gastrointestinal system, with symptoms

including stomach cramps, nausea, and diarrhea. For people like the elderly, young children, or people with a weakened immune system, this illness can become serious and even lead to death (Tuft University, 1998).

Carol Toker Forman, former Assistant Secretary of Agriculture, has brought the issue of food contamination caused by processed chicken to the Senate subcommittee (The Consumer Medical Journal, 1991). She has been trying to get Congress to require food processors to provide handling instructions on their packaged chicken. This would require every package to have written instructions on how to properly prevent cross contamination. She found that one of the biggest problems with cooking chicken in domestic kitchens was that consumers did not know how to properly handle chicken without contaminating everything in their kitchen. She concluded that the improper handling of chicken is the cause of the increase in food poisoning in the United States (Consumer Medical Journal, 1991). Anything that contaminated chicken touches becomes

contaminated. These contaminated surfaces include cutting boards, sponges, sinks, cooking utensils, counters, drawer pulls, and other foods like salads (Consumers Medical Journal, 1991). When a domestic kitchen is not properly disinfected, it can lead to harmful bacteria that can cause food borne illness.

It has been found that many of the cleaning practices people use to clean their kitchens are actually making their kitchens dirtier. Sponges and rags are commonly been used to wipe up spills and clean dishes, but it has been found that in domestic kitchens, sponges and rags actually spread bacteria rather than disinfecting it (Gallo, 1998). Many people don't disinfect their sponges and rags after every use. This is a problem because bacteria lurk in sponges, dishrags, and everywhere the bacteria are spread, until they have been properly disinfected. Sponges need to be put in the dishwasher, and rags need to be put in the washing machine. After a few weeks, both should be discarded (Gallo, 1998).

A study done by Raloff (1996) tested the spreading of bacteria by sponges and dishrags. Different household kitchens were tested once a day for bacteria for a period of one week. Large amounts of bacteria were found on dishrags and sponges that were collected from the homes. Pathogenic bacteria were found on dishrags and sponges, including *E. coli* and strains of *Salmonella*, *Pseudomonas*, and *Staphylococcus* (Raloff, 1996). Large amounts of contamination were also found throughout the kitchen on places like the counter tops and refrigerator handles. On the sixth day of collecting bacteria, one of the kitchens being tested was found to be nearly germ free, this was because the family had started using a new sponge. This study found that sponges and dishrags are ineffective when it comes to cleaning up messes if they are not germ free themselves.

Another study was done by Barker and his colleagues (2003) to test the most effective way to kill *Salmonella* bacteria by using different washing practices. He and his colleagues set up a model kitchen to test the spread of bacteria from a contaminated piece of chicken and how to properly disinfect the contaminated places. Contaminated chicken was placed on a counting board and cut with a knife. The contaminated hands used to prepare the chicken then touched a refrigerator, telephone, and a dishcloth. Swabs were taken from each of the contaminated places to test for bacteria; these were the control groups. Then, the kitchen was cleaned in several different ways. First the cutting board, knife handle, telephone receiver handle, refrigerator handle, and the work surface were cleaned with the bowl washing method, in which a dishrag soaked in a bowl full of warm water and dish soap was used to clean the surfaces. Next, the same surfaces were contaminated the same way, but cleaned differently. The knife, dish cloth, cutting board were all wiped with the bowl washing method, except they were all rinsed in cold water for 20 seconds after being wiped with the dishrag. The dishrag was then used to clean the counter tops, telephone, and refrigerator handle. Once again, the surfaces were contaminated the same way, but cleaned differently. All the objects were cleaned again with the bowl washing method, but after the objects were washed, they were sprayed with a disinfectant.

The results of this experiment showed that when handling contaminated chicken, the spreading of the bacteria was consistent throughout the kitchen each time chicken was handled and prepared. It was found that surfaces that were in direct contact with the chicken had 100% contamination and the surface, not in direct contact also had approximately 100%

bacterial contamination. When the surfaces were cleaned with the bowl washing method, 96.6% of bacteria were still found and when the surfaces were cleaned with the bowl washing method and rinsed, 93.3% of the bacteria were still found on the surfaces. The surfaces that were washed, rinsed, and had disinfectant used on them had only 6.6% contamination remaining. This study found that rinsing was a critical step in achieving hygiene, but to achieve 93% reduction of bacteria, disinfectant products were needed (Barker 2003).

*Salmonella* and *Campylobacter* bacteria are bacteria found in many poultry products, and are the two most common bacteria found in domestic kitchens (Barker, 2003). People are more likely to contract a food borne infections in domestic homes than from eating in restaurants, cafes, and bars (Schmidt, 1998). This is because people who work at these establishments have training on how to disinfect properly, unlike most users of domestic kitchens. This is a problem because when kitchen bacteria are not disinfected properly, they can be dangerous. Bacteria can cause intestinal infections, which cause a person to have flu like symptoms. Each year millions of children and adults get food poisoning and at least 9,000 people a year die from it (Franklin, 1998).

Bacteria is found everywhere in homes, but it is most abundant in kitchens. Kitchens are where most food preparation takes place, so foods that spread bacteria, like raw meats, are constantly contaminating kitchens with bacteria. According to the Food Safety and Inspection Services (2003), chicken is the most consumed meat in America. With this statistic, it means that one of the most common types of kitchen bacteria is *Salmonella*. Each time raw chicken touches a surface, that surface needs to be disinfected to prevent bacteria from growing and spreading. Since *Salmonella* is

microscopic, it cannot be visually detected by eye.

Kitchen bacteria are like any other bacteria; they grow in moist, dark climates. This includes dishrags, sinks, wet countertops, or cutting boards, and refrigerators. Without proper disinfections, people can contract food borne illnesses and become very sick (Tufts University, 1998). Bacteria are easily spread from contaminated knives and cutting boards to the hands and mouth of a person. It is important that proper disinfectant techniques are used while handling chicken, so bacteria are not spread.

In the United Kingdom, Cogan (2001) performed an experiment to test chickens for bacteria and to test kitchens for bacteria when preparing raw chicken. Cogan (2001) had a group of volunteers prepare chicken for cooking in a model kitchen. The volunteers were given different instructions on how to prepare the chicken. The instructions varied from what they touched with contaminated hands to how they disinfected their work surface and utensils. Before the chicken was prepared for cooking, each chicken was tested for bacteria. The study found that 31-41% of chicken prepared was contaminated with *Salmonella* and more than 80% was contaminated with *Campylobacter*. In the UK, it has been reported that more than 1260 tons of chicken is consumed per day; with these results it would mean that 1 in 25 families prepare contaminated chicken each day. Cogan (2001) also found that bacteria from contaminated chicken could be spread from hands to kitchen surfaces during preparation.

Many people use soap and water to disinfect their kitchens, but this has been shown to be ineffective (U.S. Department of Health, 2002). The U.S. Department of Health has found that soap and water reduces the appearance of dirt, but does not kill many strains of bacteria. According to

the FDA, the most effective way to kill bacteria is to use commercial disinfectants after using soap and water. This will prevent the spreading of the bacteria.

Disinfectants are specifically designed to kill bacteria, viruses, and mildews that are found on kitchen and bathroom fixtures, and flourish on counters and drain gates in sinks (Franklin, 1998). Using disinfectants is important in households, because they prevent the spread of bacteria and the contraction of food borne illnesses. These disinfectants must be used properly or they can end up affecting the person doing the cleaning more than the bacteria being disinfected. When disinfectants are used, they need to be used as directed in order for them to be effective. Rubber gloves must be worn to protect hands, the room needs to be well ventilated to protect the lungs, and if there is thick amount of grime, it must be scrubbed first and then sprayed with disinfectant (Franklin 1998).

These previous studies show the importance of using disinfectants to kill kitchen bacteria. My experiment tested different kitchen cleaners to determine which are the most effective, so it was important for me to know which bacteria are most commonly found in kitchens and how they are spread around the kitchen. The purpose of this study was different from the previous studies, because I was trying to find which name brand products are the most effective in killing household bacteria. Many of the previous studies tested active ingredients found in these products, but none of them told consumers which product is the most effective. I am assuming that when most consumers are at the store buying cleaners, they don't look at the back of the bottle to find and compare active ingredients; they look at the front of the bottle. As a consumer, I look at the colorful labels and a brand name, not ingredients. It

is important for consumers to know which brand of disinfectant is most effective, so they can buy products that they know are going to disinfect their homes and keep them healthy.

I studied the effectiveness of kitchen disinfectants against the *Salmonella* bacteria. I tested 6 different disinfectants sprays and wipes to determine which was the most effective against this type of bacteria. I tested my hypothesis by growing *Salmonella* bacteria in Petri dishes and then using the different disinfectants to see which were most effective at killing the bacteria. I did this by measuring the zone of inhibition for each disinfectant. This experiment is important because it will find the most effective disinfectant, which will then help consumers buy the best kitchen cleaner. My hypothesis was that Clorox Spray and Wipes would be the most effective disinfectants.

## Methods

In this experiment, many steps were required in order to find the most effective disinfectant needed to kill salmonella bacteria in household kitchens. The disinfectant products that were used were tested the same way, except two different incubation times were used. The first way the disinfectants were tested was filter paper disks were placed on four quadrants of the Petri dishes containing the *Salmonella* bacteria. The disinfectants were then applied to each filter disks and then incubated for 24 hours. This was then repeated a second time, except the dishes were incubated for 48 hours.

To begin this experiment, *Salmonella* bacteria were grown. The reason for choosing *Salmonella* bacteria was because chicken is one of the most consumed meats in the United States and *Salmonella* is commonly found in chicken. Each disinfectant was tested a total of 24

times, twelve tests at 24 hours and twelve tests at 48 hours, using zone of inhibition.

### Preparing Agar for Petri Dishes

Before the Petri dishes were inoculated with the *Salmonella*, nutrient agar was prepared to pour in the bottom of each dish. To make the nutrient agar plates, 20 g of Bio Pro Premium nutrient agar powder (final concentrations of 3g/L beef extract and 5g/L bio gel peptone) was mixed with 250 mL of water in a 2 L flask. The flask was then placed on a hot plate and boiled for 1 minute while the mixture was stirred. The mixture was then poured into smaller flasks and autoclaved in a Barnstead autoclave at a pressure of 15 psi. The flasks were autoclaved at 120°F for 20 minutes. After it was autoclaved, the agar was cooled to 55° C before pouring into the Petri dishes. Once the agar was cooled, a surface was bleached to sterilize the surface on which the Petri dishes were placed. Approximately 15 mL of the agar solution was poured into the bottom of each of the 38 Petri dishes. Each dish was cooled for 20 minutes and then wrapped and placed in the refrigerator for storage.

### Growing *Salmonella*

To grow the *Salmonella*, 1 mL of nutrient broth was pipetted onto the 100% concentration of *Salmonella* and was mixed. After the *Salmonella* was mixed with the nutrient broth, 1 mL of the mixture was removed and placed back into the original stock tube containing the nutrient broth. The mixture was then incubated for 24 hours at 37°C. After the *Salmonella* was incubated for 24 hours, each dish was inoculated with 100 µL of liquid *Salmonella* from the stock tube. After the *Salmonella* were placed on the plate, they were spread over the plate with a glass rod. The plates were then ready to have the disinfectants applied.

### Applying Disinfectants to *Salmonella*

The first set of disinfectants that was tested was the sprays. These sprays were Clorox Disinfecting Spray (Sodium Hypochlorite 1.85%), Lysol Disinfecting Spray (Alkyl (67% C12, 7% C16, 1% C8-C10-C18) Dimethyl benzyl ammonium chloride 0.08%, Alkyl (50% C14, 40% C12, 10% C16) Dimethyl benzyl ammonium chloride 0.02%), and Safeway Orange Spray (no active ingredients listed). Before disinfectants were applied to the Petri dishes, filter paper was hole-punched and autoclaved. Once the filter paper disks were autoclaved, one disk was placed on each of the four quadrants of 18 contaminated Petri dishes, by flame sterilizing a pair of forceps between the application of each disk. The disinfectant sprays were then applied. Each liquid disinfectant was placed in a sterile Petri dish, and then 5 µL of each disinfectant spray was micropipetted on to each disk. Each disinfectant spray was added to a total of 6 dishes or 24 disks. Three dishes were incubated for 24 hours, and the other three were incubated for 48 hours.

This procedure was then repeated for the three types of wipes being tested, which were Clorox Disinfecting Wipes (n-Alkyl (60% C14, 30% C16, 5% C12, 5% C18) Dimethyl Benzyl Ammonium Chloride 0.145%, n-Alkyl (68% C12, 32% C14) Dimethyl Ethylbenzyl Ammonium Chloride 0.145%), Lysol Disinfecting Wipes (Alkyl (50% C14, 40% C12, 10% C16) Dimethyl benzyl ammonium chloride 0.28%), and Safeway Brand Wipes (n-Alkyl (68% C12, 32% C14) dimethyl ethylbenzyl ammonium chlorides 0.14%, n-Alkyl (60% C14, 30% C16, 5% C12, 5% C18) dimethyl benzyl ammonium chlorides 0.14%, Isopropyl alcohol 8%). Liquid from each of the wipes was extracted into a sterile Petri dish while wearing sterile gloves. The sterile disks were placed onto the four

quadrants of 18 Petri dishes that had been inoculated with *Salmonella*. The disinfectants were then applied to the disks. Five microliters of each disinfectant wipe was micropipetted on the disks. Each disinfectant wipe was added to a total of 6 dishes or 24 disks. Three dishes of disinfectant were incubated for 24 hours, and the other three were incubated for 48 hours.

There were also control groups that were inoculated. Two Petri dishes were contaminated with *Salmonella*, had sterile disks placed on them, but instead of a disinfectant being placed on the disks, 5  $\mu$ L of sterile water was micropipetted onto them.

### Measuring Zone of Inhibition

After the 24-hour or 48-hour incubation time, the dishes were observed. The zones of inhibition were measured by measuring the diameter of the clear inhibition zone surrounding each disk with a ruler.

### Statistical Analysis

A statistical analysis was performed after collecting the zone of inhibition measurements. An ANOVA statistical analysis was performed. The program that was used to statistically analyze the data was Minitab Release 14 Statistical Software (Minitab Inc., 2005). An ANOVA tests the difference among the means of two or more groups. An ANOVA test and Tukey tests were done for all the zone of inhibition measurements for the 24-hour incubation time and for the zone of inhibition measurements for the 48-hour incubation time. This statistical analysis showed which disinfectant was the most effective in killing the bacteria. The disinfectant that had the largest zone of inhibition was the most effective.

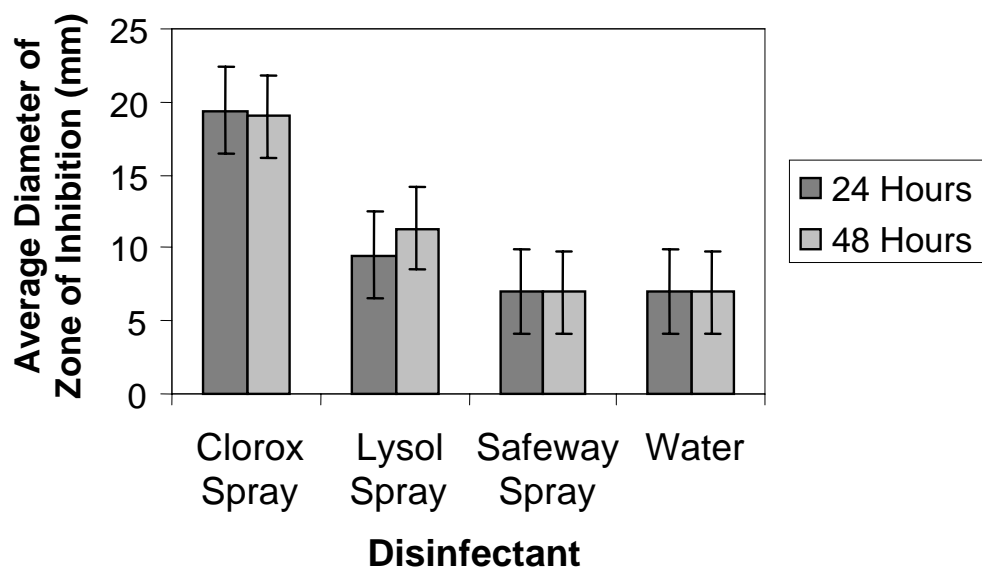
## Results

### Effectiveness of disinfectant sprays after 24 and 48 hours:

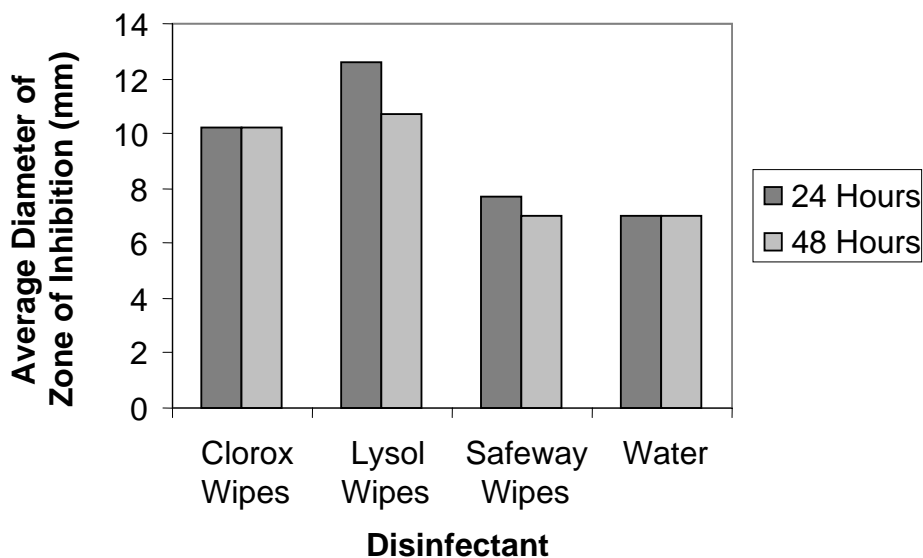
Experiments were performed with six disinfectants against the bacteria *Salmonella*. Lysol, Clorox, and Safeway brand disinfectants of both wipes and sprays were tested against *Salmonella* for 24 and 48 hours. An ANOVA test was performed to statistically analyze the data. The results of the ANOVA conducted on the disinfectants after a 24 hour incubation showed that the disinfectants did not all have the same ability to kill *Salmonella* ( $f = 43.88$ ; d.f. = 5;  $P < 0.0001$ ). A Tukey test showed that Clorox spray was significantly different from the other disinfectants (individual confidence level 99.54%).

Clorox spray had a great effect on *Salmonella* bacteria after being incubated for 24 hours (Figure 1). It had the large average zone of inhibition of 19.4 mm. Lysol wipes after the 24 hour incubation was found to be significantly different from Safeway wipes, but not from Clorox wipes (Figure 2).

After the disinfectants were incubated for 48 hours, an ANOVA showed that there were differences among the disinfectants ( $f = 107.46$ ; d.f. = 5;  $P < 0.0001$ ). Tukey tests showed that Clorox spray was again significantly different than the other disinfectants and that Clorox was the most effective disinfectant spray and the most effective disinfectant overall (individual confidence level 99.54%). For the disinfectant wipes, Lysol was found to be more effective than Safeway wipes but not more effective than Clorox wipes (Figure 2).



**Figure 1:** Comparison of average zone of inhibition values for 24 hour and 48 hour incubated disinfectant sprays for 12 trials for each disinfectant. Clorox spray shows to have the largest average zone of inhibition followed by Lysol spray and then Safeway spray.



**Figure 2:** Comparison of average zone of inhibition values for 24 hour and 48 hour incubated disinfectant wipes for 12 trials for each disinfectant. Lysol wipes shows to have the largest average zone of inhibition followed by Clorox wipes and then Safeway wipes.

## Discussion

The results of my experiment did not fully support my hypothesis that the most effective disinfectants would be Clorox spray and Clorox wipes. Studies have been done which tested disinfectants against *Salmonella* (Barker 2003). However, no experiments have been done that tell the consumer which products are the most effective at killing *Salmonella*.

I found statistically that Clorox spray was most effective of all the disinfectants (Figures 1 and 2) in eliminating *Salmonella* as measured by zone of inhibition after being incubated for 24 and 48 hours. Lysol wipes were found to be more effective than Safeway brand wipes, but not more effective than Clorox wipes.

These results suggest that differences in active ingredients of the disinfectants that were tested could be working through different mechanisms to eliminate bacteria. The effectiveness exhibited by the different disinfectant chemicals could be different, which in turn could cause differences in the zones of inhibition. Although the mechanistic actions of the disinfectants were not explored in this experiment, I can conclude from my conclusion that the Clorox spray was better able to kill *Salmonella* than the other disinfectants.

Despite the fact that Clorox was the most effective disinfectant spray, the Clorox brand wipe was not the most effective disinfectant wipe (Figure 2). This may have been because Clorox spray contained the active ingredient sodium hypochlorite and the Clorox wipes did not contain this active ingredient. Since Clorox spray was found to be most effective disinfectant and it was the only disinfectant that contained Sodium hypochlorite, it is likely that sodium hypochlorite is the best active ingredient at killing of the *Salmonella* bacteria.

I found it interesting that the Safeway orange spray was the only disinfectant that did not have any active ingredients listed on the bottle. Many consumers buy this product and, from my study, I found it to be ineffective at eliminating bacteria. It's unfortunate that this ineffective product is on the market.

In conclusion, despite the substantial evidence provided by my experimental results that Clorox disinfectant spray was the most effective disinfectants in the elimination of *Salmonella* (Figures 1 and 2), the conclusiveness of my results cannot be fully appreciated without further investigation of the active ingredients of the disinfectant products. I think that someone could expand on this study by testing the active ingredients of the disinfectants to find if sodium hypochlorite really is the most effective ingredient or if there are other ingredients in the products that are effective. Another study someone could do would be to test other types of bacteria such as *E. coli*. I also think it would be important to test viruses like the flu and cold virus against the disinfectants. This would give more information results about the effectiveness of each disinfectant.

In conclusion, from the results of my study I can highly recommend Clorox spray because it was the only disinfectant that was significantly different from the other disinfectants.

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