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Effectiveness of Antibacterial Soaps according to the Manufacturer

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ABSTRACT

Handwashing with soap and water, while an acceptable way to cleans hands of oils, fats, soil, and microorganisms, is not very effective at eliminating possible harmful bacteria that can be found on hands. In this study 6 volunteers were asked to not use any personal hygiene product containing any antibacterial agents for 1 week. After the week, each volunteer was then inoculated with *E. coli* and each hand was washed with a different soap whose manufacturer claims a 99.9% reduction in bacteria from the hands two separate times. After plating of the sampling solution, plates were incubated for 1 day (24 hours). After 24 hours, a count was conducted on the plates. Individually Lysol® and Dial® both soaps eliminated at least the stated reduction of 99.9% in which the manufacturer's claimed their products would eliminate. An overall average was taken and 2 out of 4 tests showed a 100% reduction.

Keywords: *Antibacterial Soaps, Triclosan, ASTM E2870-13, Hand Washing, Benzalkonium Chloride*

INTRODUCTION

A bacterium is a single-celled organism that can live in a variety of climates and on a variety of surfaces. Some bacteria can live harmlessly amongst a host, while others can cause the host mild to severe problems, the worst being death of the host.

In today's society, bacteria can be spread rapidly among people. Currently, food, water, hands, and surfaces are great environments for bacteria to grow. Contact with these surfaces can cause a person to get sick or spread the bacteria from person to person and surface to surface. Bacterial infections coupled with other diseases claim millions of lives each year. (Oranusi, Akande, and Dahunsi, 2013). Controlling colonization of bacteria is very difficult given there are thousands of strains and types of bacteria that live in a variety of climates and have a multitude of different needs for growth.

One way for people to control the spread of bacteria is through hand hygiene, commonly known as hand washing. Hand hygiene is the "act of cleansing the hand with water or another liquid, with or without the use of soap or other detergents, for the purpose of removing soil, dirt, and/or microorganisms" (Oranusi, Akande, and Dahunsi, 2013). Currently, hand hygiene is the easiest, and most cost-effective way to reduce spreading of potentially deadly microorganisms.

While technical definition of hand hygiene includes water as a source of cleansing, water alone is ineffective at removing or killing bacteria and other microorganisms. Water typically cannot remove the oils, fats and proteins on skin that give a good environment for some bacteria to grow. A slightly more effective method includes using liquid or bar soap that does not contain any antibacterial components. While the soap helps remove the fats, oils, and proteins better than water alone, it still leaves environments suitable for bacterial growth. By current standards, water and soap are acceptable ways of reducing the spread of bacteria from person to person and surface to surface (Oughton, et al. 2009; Jabbar, et al. 2010). Medical facilities, such as hospitals and doctor offices, take extra care in making sure they are decontaminated including under and around fingernails, cuts or scrapes and jewelry. While natural nails are excellent places for bacteria to grow, artificial nails have been shown to harbor more bacteria than natural nails (Griggs, 2010). Also another highly contaminated place one needs to be aware of are bulk soap dispensers typically found in public restrooms. One in every four dispenser has found to be contaminated with bacteria and pathogens (Zappa et al., 2011).

Currently, the best method for removing dirt, soil, and/or microorganisms from skin includes use of water along with a soap which holds antibacterial properties which have the ability to eliminate the bacteria from the skin. Triclosan is a phenoxyphenol antimicrobial that is used in hygiene products (Aiello, Larson, and Levy, 2007). Triclosan inhibits the growth of gram-positive and most gram-negative bacteria. A few bacteria Triclosan is ineffective at inhibiting growth include *Pseudomonas aeruginosa* and *Serratia marcesens* (Aiello, Larson, and Levy, 2007). *P. aeruginosa* and *S. marcesens* become resistant to Triclosan when mutations occur at binding sites where the Triclosan would penetrate the bacteria. At high concentrations, Triclosan is considered a bactericide and can be used successfully as a disinfectant on surfaces including hands. At lower concentrations, Triclosan becomes a bacteriostatic which inhibits the spread and growth of bacteria but does not kill the bacteria. Triclosan not only kills and eliminates bacteria, it also has some antiviral and fungal activity (Aiello, Larson, and Levy, 2007).

Benzalkonium chloride, BC, is a quaternary ammonium compound commonly used as a disinfectant. BC's low toxicity makes the compound widely effective over a wide pH scale (Bore, et al.,

2007). BC is commonly used to care for cuts, burns, and minor abrasions of the skin. BC helps cleanse wounds of any bacteria that could have been transferred or settled into the wound.

This study will concentrate on the use of antibacterial soaps containing Triclosan and Benzalkonium Chloride and their effectiveness at eliminating *Escherichia coli*. Many manufacturers claim their products kill 99.9% of bacteria that are currently on the skin. Lysol® claims their liquid antibacterial hand wash kills 99.9% along with Dial® whom also make the same claim with their Complete foaming soap (Lysol®, 2013; Dial ®, 2013). How can we as consumers be sure what they are stating on their product or their website is what their product actually does? While every product has to go through standard testing before being put on the market, is the claim by the manufacturer the actual results found during testing?

MATERIALS AND METHODS

The methods and materials used are those set by the ASTM in E2870-13 for testing of Evaluation Relative Effectiveness of Antimicrobial Handwashing Formulations using the Palmar Surface and Mechanical Hand Sampling (ASTM, 2013). A summary of procedures is as follows: Each of the 6 volunteers spent a week prior to testing using Triclosan, BC and other antibacterial agent free products. These products included but were not limited to shampoo, conditioner, toothpaste, body wash, deodorant, and hand soap. After a week of using the products, each participant was given a thorough examination of their hands for any cuts, hangnails, abrasions, burns, and or open wounds. If the volunteer does have any breaks in the skin on their hands they will be excused from testing. All volunteers without breaks in the skin will have 100μL of *E. coli* added to each hand and asked to carefully rub it across their palms only. Each hand was then washed using a specific soap that states by their manufacturer kills or reduces bacteria by a certain percent. After the washing of the hands, each hand was placed into bag containing a sampling solution, secured at the wrist and placed on top of a piece of carpet. The volunteer pressed down slightly on the carpet through the bag and rub back and forth for 3 minutes. After the 3 minutes, the solution in the bag was collected into a specific container that was plated later for growth. Each volunteer placed their hands into another bag of sampling solution and repeated the carpet procedure and the sampling solution from these bags was placed into different containers and again plated for growth. Each volunteer underwent the procedure of inoculation and washing a second time before undergoing a 2 wash sequence to ensure all *E. coli* was eliminated. After washing was completed, each beaker containing the sampling used in the bags were plated. 100μL of sampling solution was placed on a plate containing Levine EMB Agar. Each plate was incubated at 37 ± 0.5 C for 24 hours and then removed from the incubator counted, photographed and returned to the incubator to have the counts and photographs repeated at 48 hours and 72 hours.

Changes to the ASTM E2870-13 are listed below referenced by their step number.

6.3: Reference to the use of Shaker Incubator, a regular incubator that does not have the capability of shaking was used instead.

6.4: For sterilization of broth, agar, and sampling solution, the use of an autoclave was used.

6.7: A vortex mixer was not be used as I did not centrifuging *E. coli* solution.

6.8: In reference to the use of Mechanical Scrubber, pieces of carpet were used. Friction that the mechanical scrubber would supply, participants were instructed to press with constant pressure down onto the carpet to where they can move their hands back and forth and not wear a hole into the bag. Procedure lasted 3 minutes.

6.12-13: A centrifuge apparatus and tubes were not used as I have decided not to centrifuge the sediment.

6.16: Plastic bags suggested at a size of 30x18 centimeters were not used. In their place I used an autoclavable bag that is 25x25 centimeters in size.

6.17: Suggestion of a tourniquet of some kind, Velcro straps were used to tie the plastic bag around the volunteer's hands during testing.

7.3: For the dilution fluid, an equivalent diluent was used and 0.1 M HCL and 0.1 M NaOH was used to adjust pH.

7.4: In place of Soybean-Casein Digest Agar with MUG, Levine EMB Agar was used. Levine EMB Agar will allow for and *E. coli* to be easily seen and counted.

7.5: In place of Soybean-Casein Digest Broth, Tryptic Soy broth was used to support the growth of *E. coli*.

7.6: In place of Soybean-Casein Digest Agar, nothing was used. Since I used the Levine EMB agar, it will support the growth of the *E. coli* and also serve the purpose of allowing for easy counting.

7.9: For the Chlohexidine Gluconate 4% Solution/Antiseptic/Antimicrobial Skin Cleanser, a Walgreens Antiseptic Skin Cleanser was used.

8.1: For the *Escherichia coli* ATCC 10536, *Escherichia coli* K-12 strain was used in its place.

10.6.3: No mechanical scrubber was used. As seen in 6.8, pieces of carpet were used in its place.

10.6.6: Upon recovery, each volunteer repeated the process a second time starting from 10.2 and ending with 10.6.6.

Each volunteer underwent the procedure twice and a total of 6 plates were obtained. The plates included 1 before and 1 after showing the presence of *E. coli* at

the time of testing, 1 for the initial testing of Lysol®, 1 for the initial testing of Dial®, 1 for the second testing of Lysol® and 1 for the second testing of Dial®.

In order to find out the percent reduction for each volunteer's test, concentration of *E. coli* needed to be found. To do this, I took 1mL of stock *E. coli* and placed it into a vial that contained 9 mL of Tryptic Broth. This was vortex and allowed to sit for 5 minutes. After the 5 minutes, 1mL of broth was removed from this vial and placed into another vial containing sterile Tryptic Broth. Again it was vortexed and allowed to set for 5 minutes. This was done a total of 8 times. After the 8^{th} dilution, 100μL was plated and allowed to set for 24 hours. 8 hours after the dilution of *E. coli* was made it was used on each volunteer. To take into account for growth time while broth was incubated, for every hour the broth was sitting allowing growth of bacteria, the number of colonies formed on plate made immediately after 8^{th} dilution would be times by $2^{3 \times \#\text{ of hours sitting}}$ For example: after 24 hours of incubation, 52 colonies formed on the plate. 52 would then be multiplied by 2^{3x8} for the first volunteer of the day. After 24 hours of incubation, each plate was counted, photographed and put into a spread sheet. The plate counts where then converted from the amount of bacteria in 100μL of sampling solution from the bags into number of bacteria that would be found in the entire 75mL. Once CFU was found for entire 75mL, a percent reduction calculation was done. ((CFU-Concentration)/concentration) x 100%. Lastly an average of all the reductions were then calculated for each soap for each test trial.

RESULTS

On completion of the testing, both soaps used passed with at least a 99.99% reduction of bacteria from the volunteers hands. While most reductions we seen with no bacteria growth, a few had a small amount of growth but with the large amount of *E. coli* that was present in the tube used to inoculate each volunteer, did in fact show the claim by both Dial and Lysol on the packaging of their products is in fact accurate.

The data for Dial can be found on Tables 1 and 3. Both tables show each volunteer, their sex, CFU counts and the percent reduction after the 24 hour period. Both trials for Dial showed similar reductions for each person. There was some difference between first test and second test on the effectiveness of cleansing the bacteria but not enough for there to be anything less than a 99.9% reduction.

The data for Lysol can be found on Tables 2 and 4. Similar to the Dial, Lysol too was very successful in eliminating at least 99.9% of the bacteria in accordance to the manufacturers claim on the packaging.

Table 1. This table represents the Dial data for the first round of testing. The I stands for the volunteers, 24 hour CFU is the count converted to what the entire sampling solution (75mL) would possibly produce had it all been plated, and 24 Hour Red stands for the % reduction compared to the original concentration of the E. coli. Ave stands for the average reduction seen for each the 24 hour period. To get the proper counts for CFU columns, take the number × 10^4. Example: 18 × 10^4=180000 which gives total number of colonies possibly seen if all 75 mL was plated.

I	Gender	24 Hour CFU	24 Hour Red
1 L	F	0	100
2 L	F	0	100
3 L	F	0	100
4 L	M	0	100
5 L	M	9	99.99
6 L	M	0	100
Ave.			99.99

Table 2. This table represents the Lysol data for the first round of testing. The I stands for the volunteers, 24 hour CFU is the count converted to what the entire sampling solution (75mL) would possibly produce had it all been plated, and 24 Hour Red stands for the % reduction compared to the original concentration of the E. coli. Ave stands for the average reduction seen for each the 24 hour period. To get the proper counts for CFU columns, take the number × 10^3. Example: 18 × 10^4=180000 which gives total number of colonies possibly seen if all 75 mL was plated.

I	Gender	24 Hour CFU	24 Hour Red
1 R	F	0	100
2 R	F	0	100
3 R	F	0	100
4 R	M	0	100
5 R	M	6.750	99.99
6 R	M	0	100
Ave.			99.99

Table 3. This table represents the Dial data for the second round of testing. The I stands for the volunteers, 24 hour CFU is the count converted to what the entire sampling solution (75mL) would possibly produce had it all been plated, and 24 Hour Red stands for the % reduction compared to the original concentration of the E. coli. Ave stands for the average reduction seen for each the 24 hour period. To get the proper counts for CFU columns, take the number × 10^3. Example: 18 × 10^3=18000 which gives total number of colonies possibly seen if all 75 mL was plated.

I	Gender	24 Hour CFU	24 Hour Red
1 2L	F	2.4	99.99
2 2L	F	36.75	99.99
3 2L	F	0	100
4 2L	M	0	100
5 2L	M	0.75	100
6 2L	M	0.75	100
Ave.			100

Table 4. This table represents the Lysol data for the second round of testing. The I stands for the volunteers, 24 hour CFU is the count converted to what the entire sampling solution (75mL) would possibly produce had it all been plated, and 24 Hour Red stands for the % reduction compared to the original concentration of the E. coli. Ave stands for the average reduction seen for each the 24 hour period. To get the proper counts for CFU columns, take the number × 10^3. Example: 18 × 10^3=18000 which gives total number of colonies possibly seen if all 75 mL was plated.

I	Gender	24 Hour CFU	24 Hour Red
1 2R	F	2.25	99.99
2 2R	F	0	100
3 2R	F	0	100
4 2R	M	0.75	100
5 2R	M	0.75	100
6 2R	M	0	100
Ave.			100

DISCUSSION

It is becoming more common in any hygiene product being bought for it to contain Triclosan, BC or any other antibacterial agent in everyday products. The use of products with antibacterial agents, increase the elimination of bacteria compared to water or soaps which do not contain antibacterial agents plus water (Oranusi, Akande, and Dahunsi, 2013). Without searching on the shelves of a retail store, it is hard to find products that do not contain any antibacterial agent.

After testing, it shows both manufacturers' are in fact claiming the correct amount of reduction. Both Dial and Lysol have claimed that the products that were tested during this experiment reduced or killed 99.9% of bacteria (Dial®, 2013; Lysol®, 2013). Individually all 24 tests showed at least 99.99% reduction and collective average show 2 tests had 100% elimination and the other 2 had 99.99% reduction.

During testing, it was found that the foam soap was easier to get off of the skin as well as feel as though the hands were completely covered in soap before rinsing.

Also seen was the difference between males and females. Collectively the females had more bacteria growth than that of the males. While all volunteers had to keep nails short in accordance to the experiment design, longer nails will typically hold bacteria underneath them and make it harder to get completely off (Griggs, 2010).

Another thing to take into consideration is the type of work each volunteer does. One of the male volunteers used in testing is an Auto Restoration Major while the majority of the other volunteers were Science Majors. As Science Majors, depending on the class, we are exposed to bacteria at least once during the course of a week of school. While the Auto Restoration is exposed to grease and other oil and grease based products along with the constant use of tools had dried out his hands and caused calluses. This volunteer was the only one to show bacteria growth during each test concluding that a difference in skin types can cause bacteria to hide within the calluses and roughness of the skin.

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