Reduction of Hand Bacteria: A Comparative Study among Common Antiseptics

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Abstract

The efficiency of common household hand sanitizers containing several antiseptics was assessed in this experiment. Two different hand washing protocols were tested: antiseptic hand gels that do not require the use of water and antiseptic hand soaps that require the use of water. Antiseptic gels investigated included Germ-X Original Hand Sanitizer, Purell Hand Sanitizer, and Wet Ones Hand Sanitizer. Antiseptic soaps included Softsoap Antibacterial Liquid Hand Soap, Dial Complete Foaming Handwash, and Trader Joe’s Kitchen Anti-Bacterial Hand Soap. Pure tea tree oil was also investigated. Two samples were taken from each participant: an initial sample of hand bacteria was acquired before antiseptic treatment via sterile swab, and a second sample was acquired after antiseptic treatment. The greatest bacterial reduction appeared after treatment with Wet Ones Hand Sanitizer (55.56% reduction). A one-way analysis of variance (ANOVA) test revealed no statistically significant difference when comparing bacterial reduction among products (p = 0.106; d.f. = 55). A second ANOVA test involved a comparison between antiseptics gels, soaps and tea tree oil. This test revealed no statistically significant difference (p = 0.32; d.f. = 55).

Introduction

Although the importance of hand washing is generally accepted as a preventative measure to decrease transmittance of disease, a study conducted by The New England Journal of Medicine in 1992 reported hand washing compliance was only 30%-48% in an intensive-care unit (Case, 2006). The U.S. Food and Drug Administration (FDA) has set up a system of loose standards for effective antiseptic products, but there is no legally binding document that forces manufacturers to comply. Throughout the past thirty years or so, the use of antiseptic products, in and out of the healthcare system, has increased. Many consumers place trust in antiseptics everyday, but how effective are these sanitizers?

Products containing antimicrobial agents that kill, inhibit or reduce the number of microorganisms on the skin are topical antiseptics (Jackson, 2005). Although normal flora can display agonistic affects, where one organism forms a symbiotic relationship with another organism, the flora may also serve as a source of infection for the host. There are two types of normal flora on the skin: transient and resident flora. Resident flora can be persistently found on the skin, while transient flora are contracted from the external environment (Jackson, 2005). The current standards for antiseptic products only require the elimination of transient microorganisms.

Antiseptic products have been found to eliminate bacteria in two ways. The use of active ingredients found in the actual product and the washing, rinsing and drying process help to eliminate flora on the hands. The most effective way active ingredients kill the flora is by breaking down the bacterium’s cell membrane (Jackson, 2005).
History of Regulations

A division of the FDA called the Nonprescription Drugs Advisory Committee (NDAC) has been involved in decisions concerning antiseptics. The NDAC published the first formal report discussing antibacterial products in 1974. There were two distressing issues with the use of antimicrobial soaps at that time, including that studies concerning these products were not sufficient and that there were no active ingredients for these soaps that were recognized to be safe and effective. After reviewing the recommendations in this 1974 report, the FDA published the Tentative Final Monograph (TFM) in 1978. More recently, the FDA has divided into healthcare antiseptics, food handler antiseptics and consumer antiseptics. It has also been decided that all antiseptic products that include antimicrobial labeling, i.e. kills the germs that cause body odor, are drugs and are required to demonstrate effectiveness (NDAC, 2005b).

Recently, in vitro and in vivo studies have tested the reduction of transient bacteria. In vitro studies observe the number and movement of organisms as well as the potential for the development of resistance (NDAC, 2005). In vivo test methods look at other aspects, such as patient-to-patient contamination, and whether or not there is adequate bacterial reduction through tests that mimic actual use. Hands are contaminated, washed, and then the number of flora are noted.

Efficacy of antiseptics

Within all antiseptic products, there is an active chemical agent (called a biocide) responsible for the destruction of microorganisms. These active ingredients include alcohol, iodine, triclosan, chlorohexidine gluconate, benzalkonium chloride, benzethonium chloride, triclocarban, and benzethonium chloride, para-chloro-meta-xylenol, triclocarban, and triclosan (NDAC, 2005b). Leave-on handwashes contain alcohol, benzalkonium chloride, and benzethonium chloride. Yet, although all of these biocides may be used by manufacturers, only two active ingredients have been recognized as safe and effective by the TFM. These active ingredients are 60-95% alcohol and 5-10% povidone-iodine (NDAC, 2005b).

Researchers are still working towards a conclusion on which method of antiseptic use is most effective, hand gels that do not require water or soap and water. In a recent amendment to the TFM, it was established that ethanol (60-90%), an active ingredient in hand gels, fell into Category I: safe and effective (Jackson, 2005). Although, if the hands are heavily soiled, it is not suggested that alcohol-based products replace regular soap and water. In response to a study done by Sickbert-Bennett (2005) stating that alcohol-based hand rubs are generally ineffective, it has been suggested that an increase in the percentage of ethanol may improve the effectiveness of these products (Steinmann, 2005). Regardless, it can be argued that in the case of heavily soiled hands, regular soap and water cannot be replaced.

Antimicrobial resistance

One of the most important issues with continuing the use of antiseptics is the potential antibacterial resistance these microorganisms may acquire. Sheldon’s (2005) article discusses how microorganisms can become resistant to the active ingredients found in antiseptic products. In vitro tests were performed to determine whether or not susceptibility is a factor. Researchers found two different ways that bacteria can become insusceptible to biocides. For intrinsic insusceptibility, the composition of the cell wall begins to deteriorate and the microorganism
undergoes physiological adaptation (Sheldon, 2005). Acquired insusceptibility to biocides occurs when the bacteria have mutated in some way. Although the insusceptibility of these microorganisms has not been directly observed at the genetic or molecular level, phenotypic observations reveal changes in the outer membrane of the bacteria. The real concern is that biocides may stop working altogether. Researchers and the FDA suggest that biocides be monitored in the future, so that if a strong resistance occurs, decisions can immediately be made on whether this substance is more of a risk rather than a benefit. In an FDA literary search, they found that other studies examining bacterial resistance (besides Sheldon’s research) revealed a reduced susceptibility to biocides as well (Sheldon, 2005).

Transmission of microorganisms

A study by Larson et al. (2005) involved the use of antibacterial products in a total of 238 households for 48 weeks. Each of these families had at least one preschool-aged child. To ensure their eligibility, participants were asked to complete a 31-page Home Hygiene Assessment with questions relating to demographics and illness before they began the experiment. All subjects were asked to use the products on a regular basis and the assessment was repeated every three months. Of the household members, 51.9% were 19 years of age or younger, 98.3% were Hispanic, and 53.2% were born outside of the United States. Most (83%) were in good health, while 12.1% had one or more chronic conditions. The majority of families lived in large, multiple-unit buildings (Larson, 2005). The families were randomly assigned to two groups, one using antibacterial products and the other using nonantibacterial products. The products used during this study were readily available over the counter, and all were representative of a certain type of antibacterial product such as antibacterial sprays or antibacterial soaps. All participants were given products either labeled with the term “antibacterial” or “disinfectant,” yet only the antibacterial group received the actual product. The researchers conducted weekly follow-ups by telephone and monthly home visits. During these follow-ups, participants were asked how they had been using the products and if there were any signs of illness within the family. To ensure participation, products were weighed and their masses were recorded. Participants were instructed to call their interviewer if any family member had symptoms of vomiting, diarrhea, fever, sore throat, cough, runny nose, skin infection, or “pinkeye.” There was an on-call physician available to researchers to verify the symptoms presented. Larson et al. (2005) concluded that one-third of all the households had at least one member with symptoms of infectious disease monthly. There were no differences between control and treatment groups in rates of symptoms (33.1% and 32.3% in the antibacterial and non-antibacterial groups). The most interesting conclusion was that participants with chronic disease were more likely to have fever, cough, and runny nose if they were using the antibacterial products rather than the nonantibacterial ones. This study concluded that antibacterial products did not reduce the risk of viral infections and, if a person was chronically ill, antibacterial products could increase their susceptibility to other illnesses. Even so, Larson et al. (2005) felt that antiseptics and personal hygiene in general could aid in the reduction of bacteria and the transmission of disease. Other alternatives, such as natural based products, are being considered for future use in antiseptic products (Messager et al., 2005).
Antiseptic agents can damage the skin. When the skin is damaged, there is a change in microbial flora, bacterial shedding, which can result in a greater risk of the transmission of microorganisms (Messager et al., 2005). This is especially important for healthcare workers who have to use antiseptics repeatedly as they move from patient to patient. A recent study by Messager et al. (2005), decided to take another approach to antiseptics, involving the use of natural based products, specifically examining the use of tea tree oil. There were a total of 13 participants, and each participant was asked not to use antibacterial soap or toiletries on their hands for at least 24 hours before the test. All of the subjects washed their hands with a product containing 5% tea tree oil, 60% alcohol, or nothing at all. It was found that there was a larger reduction of bacteria when tea tree oil was used in comparison to washing with regular soap or to using an alcohol-based product. Research suggests that repeated use of products containing tea tree oil does not lead to dermatological problems (Messager et al., 2005). In the future, if this type of product were to be used, there could be less drying of the skin and products like tea tree oil could encourage better healthcare staff compliance with hand washing.

Effects of Rubbing

In a study researching the antibacterial activity of several antiseptics, Messager, et al. (2004) conducted multiple tests on the effects of rubbing. A total of 15 volunteers (18-25 years of age) participated in this study. A forearm test involved the application of 20 microliters of bacteria spread in marked circles on the forearm, which were then dried for 3 minutes. Next, 30 microliters of antiseptic were spread with a sterile glass rod, leaving one circle untreated as a control. After 1 minute, a neutralizer was added and bacteria were resuspended and counted.

In a separate test, the effects of rubbing were examined by means of two different procedures (Messager et al., 2004). One version concentrated on the amount of bacteria found before and after treatment. An initial reading was taken before volunteers washed their hands for 2 minutes. After the 2 minutes of washing was completed, the second reading was recorded. In the second procedure, donated skin samples were cut with sterile scissors and mounted. One sample was mounted to a scale to determine the amount of pressure placed on the skin, and another was mounted to a mechanical drill to replicate rubbing motion of hands. The sample was then exposed to the antiseptic for 1 minute, at 200 rotations per minute, and an exerted pressure of 100 grams. Mechanical effects either produced by the volunteers washing their hands or by a drill rubbing two skin samples against each other, produced a significantly greater reduction in bacterial concentrations (Messager et al., 2004). This reveals that rubbing was an important factor in reducing bacteria.

Hypothesis

I hypothesize that antiseptic soaps, which require water, are more effective at killing bacteria than products that do not require water such as antiseptic gels or tea tree oil. The use of water and the time participants spend scrubbing allow more manipulation of the bacteria and remove it from the surface of the hands. This experiment tested 7 products which included three antibacterial soaps, three antibacterial gels, and 100% tea tree oil. Differences in bacterial reduction among products were compared by optical densities of hand bacteria found before and after antiseptic treatment.
Methods

The effectiveness of antiseptics was tested by a comparison of microorganisms found on subject’s hands before and after antiseptic treatment. Since the bacterial reduction is noted on an individual basis, differences in hand hygiene were not a factor. Participants had no limitations regarding race, gender, age, or social status. All participants acquired for this experiment were students, staff and faculty members of Saint Martin’s University. There were a total of 15 participants and 8 were randomly selected for each antiseptic test. All products, including alcohol based gels and water-based soaps, are easily accessible to the consumer. Specific products included Safeguard Antibacterial Deodorant Soap, Dial Antibacterial Soap, Trader Joe’s Kitchen Antibacterial Hand Soap, Tea Tree Naturals Antibacterial Hand Soap, Germ-X Hand Sanitizer, Purell Antibacterial Hand Sanitizer, and Hands-On Hand Sanitizer.

To test the antiseptic products, nutrient broth was used to grow bacteria obtained from the subject’s hands. To ensure there were no contaminating bacteria in the nutrient broth, the broth was sterilized by autoclaving. Nutrient broth was made by dissolving 4.8g of dry nutrient agar into 600ml of water. The nutrient broth used in this experiment was BioPro Premium Nutrient Broth, which included beef extract (3g/L) and bio-gel peptone (5.0 g/L). Four milliliters (ml) of nutrient broth was then pipetted into each of the test tubes. Then the tubes were loosely capped to allow ventilation and placed on a wire rack. The media was then placed into an autoclave (Barnstead 14-48823) with 15psi of steam pressure at a temperature of 121°C. These conditions were maintained for 15 minutes to ensure sterility. After the test tubes were autoclaved, the caps were tightened and the tubes were refrigerated until testing procedures began. Eight subjects tested one antiseptic at a time. In both hand washing procedures, areas of the hands normally missed while disinfecting were outlined in a diagram to ensure proper use of the product (Figure 1).

Figure 1. Diagram of commonly missed areas in hand washing given to participants before experimental procedures to ensure proper hand washing. Taylor. Nursing Times. 2005. http://www.healthunit.org/handwash.
A sterile cotton swab was used to swab the ventral surface of participant’s hands to obtain initial bacteria. All areas of the hands were swabbed the same way, starting with the distal end of the fingers and moving towards the thumb. In between the fingers and the palms were also swabbed to obtain an accurate sample of bacteria on the hand at that time. The handle of the cotton swab was then broken, and the swab was placed into a tube containing sterile nutrient broth. The tube was quickly capped to prevent contamination. This sample was my control, revealing how much bacteria had been acquired before the use of antiseptics. After the controls were obtained, antiseptic treatment was initiated. For antiseptic hand gels, the hands were completely moistened with the gel (1-2 ml), and all areas of the hands were rubbed until the gel evaporated. For antiseptic soaps, the hands were washed in running water at 35-45°C. Participants were asked to wash their hands for 30 seconds using 1-2 ml of antibacterial soap. The hands were then rinsed in running water and dried with a paper towel. The final test procedure involved three drops of tea tree oil (100%), which was applied via pipette to the participant’s palms. Then, participants rubbed their hands until the oil was spread evenly. After antiseptic treatment, a second swabbing procedure was completed. Samples were then incubated at 37°C for 72 hours. 37°C is the average human body temperature, so this incubation facilitated optimal growth conditions for body bacteria.

After the incubation period, the amount of bacteria from the initial sample was compared to the sample taken after antiseptic treatment. The total amount of bacteria in each sample was recorded through use of the Spectronic 20 (Bausch and Lomb) spectrophotometer. After the machine had been warmed up for 15 minutes the scale was set to record anything between 0-100% transmittance. Sterile nutrient broth was used as my control to blank the spectrophotometer, and the wavelength was set to 686 nanometers (nm). To prepare samples I used procedures outlined by Benson (2005). A monochromatic light then passed through each individual sample, which activated a photomultiplier tube on a galvanometer. When the sample yielded a low absorbance, there was a low concentration of bacterial organisms. In turn, when the sample revealed high absorbance, there was a high concentration of bacteria (Benson, 2005). The percent transmittance was recorded for each sample. To achieve a better comparison among samples, percent transmittance recording was converted to optical density using the following formula: O.D. = 100/ % transmittance. Bacterial reduction was calculated by comparing optical densities of the samples. The before O.D. was subtracted from the O.D. of samples taken after antiseptic treatment. The difference was then divided by the before O.D. to calculate the bacterial reduction. The average bacterial reduction for each antiseptic was then calculated for further comparison.

The optical density test results allowed me to compare bacterial concentrations among all samples. The optical densities for the treated samples were compared to those of the control. I used Minitab to analyze the data, including the percent reductions. T-tests compared before and after treatment groups. I calculated basic descriptive statistics (mean and standard deviation) and performed a one-way analysis of variance (ANOVA) test which tested my null hypothesis that there is no difference among the effectiveness of the different hand cleansers.
Results

The results of this experiment revealed that most antibacterial products were effective at reducing bacteria, with exception of Trader Joe’s Kitchen Antibacterial Hand Soap (Figure 2). One major observation during testing was that all products seemed to kill more bacteria when there was a higher concentration of initial bacteria. Although individual products showed reductions in hand bacteria, a one-way analysis of variance (ANOVA) test revealed no statistically significant difference when comparing bacterial reduction among products ($p= 0.106; \text{d.f.}= 55$). Therefore, individual products, before and after product application, revealed bacterial reduction yet there was no statistical difference in that reduction among products. Hands On hand sanitizer had an overall reduction of 52.47%, while Trader Joe’s Antibacterial Hand Soap revealed only a 10.31% difference before and after antiseptic treatment (Figure 3). This difference in bacterial reduction was not statistically significant ($p = 0.106; \text{d.f.}= 55$).

When comparing methods of hand washing, all methods were effective at eliminating bacteria found on the hands. The antiseptic hand gel sanitizing method revealed an average of 42.6% reduction in hand bacteria. For antiseptic soaps, there was an average of 31.11% reduction, and the tea tree oil treatment had a 27.72% average reduction (Figure 4). Although individual methods showed bacterial reductions, a comparison among antiseptics gels, soaps and tea tree oil revealed no statistically significant difference ($p= 0.32; \text{d.f.} = 55$). In other words, all methods of hand washing are effective at eliminating bacteria, but there is no difference in what method you choose to use.

![Figure 2. Differences in optical density for each sample before and after antiseptic treatment. Each treatment had a total of 16 samples; 8 before antiseptic treatment and 8 after antiseptic treatment. Error bars reveal standard deviation in percent reduction between samples.](image-url)
Figure 3. Comparison of bacterial reduction for individual products. Each treatment had a total of 16 samples; 8 before antiseptic treatment and 8 after antiseptic treatment. Error bars reveal standard deviation in percent reduction between samples.

Figure 4. Comparison of bacterial reduction for each hand washing method. A total of 48 samples, including before and after treatment, were used for antiseptic gels and soaps. For tea tree oil treatment, there were a total of 16 samples collected; 8 before antiseptic treatment and 8 after antiseptic treatment. Sanitizing gels and tea tree oil did not involve the use of water. Antiseptic soaps utilized water in the cleansing process. Error bars reveal standard deviation in percent reduction between samples.
Discussion

This study of the effectiveness of antiseptics did not support manufacturers’ claim of 99.9% reductions in hand bacteria. In fact, the greatest percentage of bacteria eliminated by a single antiseptic was just 55.56% (Figure 3). Differences in bacterial reduction among products were not statistically significant and did not support my hypothesis.

Although there was no statistically significant difference, all products and methods revealed some bacterial reduction. Overall, the antiseptic gels that did not require the use of water, killed an average of 42.6% of the bacteria. One reason could be the type of active ingredient, in this case ethyl alcohol. The use of alcohol denatures bacterial membranes to kill the bacteria present (Jackson, 2005). One observation was that although the antiseptic gels killed the bacteria present, hands that were visibly soiled remained soiled after antiseptic treatment. I believe this occurred because the product was able to penetrate into the skin, helping to lift dirt and bacteria to the surface, yet because water was not used for antiseptic gels, the dirt and bacteria was not washed away. The active ingredients for antiseptic gels included 62% ethyl alcohol for Purell and Germ X, and Wet Ones antiseptic gel contained a denatured alcohol called SD Alcohol 40, which also had 62% concentration. As stated previously, the alcohol denatures bacterial membranes, but the gel itself cannot remove substances found on heavily soiled hands (Jackson, 2005). I agree with Steinmann’s suggestion (2005) that an increase in the percentage of ethanol may improve the effectiveness of these products. The TFM (NDAC, 2005b) suggests that these products may contain anywhere from 60-90% alcohol, with most consumer products at the low end of this scale. Since many consumers are looking for a product that eliminates bacteria and moisturizes hands, manufacturers may have lowered the concentration of active ingredients to decrease drying of the hands.

For antiseptic soaps, there was an overall reduction of 31.11% (See Figure 3). Unlike antiseptic hand gel, heavily soiled hands treated with antiseptic soap did not contain visible dirt after antiseptic treatment. The use of water allowed mechanical manipulation of the dirt, penetrating deeply, releasing it from the surface of the hands. For each antiseptic soap, the active ingredient was triclosan. In comparison to other antibacterial soaps, Dial Complete Foaming Handwash showed a bacterial reduction (33.87%) and has a 0.42% concentration of triclosan. This concentration of active ingredients was much higher than the other soaps tested. Trader Joe’s Antibacterial Kitchen hand soap had a 0.20% concentration and Softsoap had a 0.115% concentration of triclosan. As one of the newest additions to the antiseptic market, the manufacturer states, “Dial Complete™ has been proven to kill both bacteria and yeast, therefore it is both antibacterial and antimicrobial” (The Dial Corporation, 2006).

One product in the antiseptic soap category only had a 10.31% reduction in hand bacteria (Figure 2). When comparing individuals tested, Trader Joe’s Kitchen Anti-bacterial Hand Soap at times increased the amount of bacteria found on the hands. This was puzzling considering that this product had more than twice the amount of active ingredient (0.2% triclosan) than Softsoap. This soap in particular contained more natural ingredients than the other soaps, including hops and rosemary. These natural ingredients may have served as a host to bacteria by providing more nutrients during the incubation process. If given a shorter incubation time, there may have been a higher bacterial reduction. Although many
suggest a 30 second hand washing period, as used in this study, this manufacturer suggests up to 2 minutes of scrubbing should occur before rinsing. This reduced hand washing time may have been a factor in the limited antibacterial effectiveness of this product.

In a study by Messenger, tea tree oil was found to rank first in comparison to all other biocides (Messenger, 2005). Although these researchers choose to test a soap containing only 5% tea tree oil, I choose to test 100% tea tree oil to examine the full potential of the biocide. Instead of a high bacterial reduction, my results concluded that there was a bacterial reduction of only 27.71%. The tea tree oil may have resurfaced dirt and oil found on the hands by penetrating into the layers of the skin. The rubbing motion may have then provided the movement needed to resurface the bacteria. It may have also allowed bacteria to grow more efficiently by providing nutrients like fatty acids. Considering many people do not use tea tree oil on a regular basis, many participants were weary about the use of this product and only three drops were applied to participant’s hands. This amount of tea tree oil may not have been adequate for antiseptic purposes.

In future testing I suggest using water as a control for a better comparison of bacterial reduction among antiseptics. I also recommend testing the effectiveness of a bleach solution on the reduction of microorganisms. Bleach has been trusted as one of the only methods to completely eliminate bacteria. Surgical scrubs and other methods used in hospital settings may provide a larger reduction in transient organisms because they contain another type of biocide called chlorohexidine gluconate. Many healthcare workers also have a much longer scrubbing time, involving a series of scrubbing techniques which would be much more effective than regular methods of hand washing.

If more data was acquired in future testing, a more accurate assessment of effectiveness could have been obtained. As many natural ingredients can provide nutrients for bacteria, the natural based products could have been given less time to incubate. This could have resulted in a larger bacterial reduction. Another factor that could be further investigated would be the effects of temperature on bacterial growth. My incubation temperature was 37°C, however a normal room temperature (20°C) may not have allowed as much bacteria to form. The humidity in the incubator may have also assisted in bacterial growth. Finally, the time spent cleansing the hands may have also been a factor in the reduction of hand bacteria. The health department, as well as other sources, suggest a 30 second scrubbing period, while others suggest a full 2 minutes. Finally, I recommend further testing on what type of bacteria is eliminated by these products. The FDA requires that transient bacteria be eliminated, yet some pathogenic bacteria may still be present.

One effective antiseptic tested was Hands On antibacterial hand sanitizer. This product is easily available to the consumer and may help aid in the reduction of transient organisms. Bacteria can be found everywhere in our environment and are capable of multiplying every 20 minutes. In fact, the number of bacteria on your body right now is greater than the number of people in the United States (Reynolds, 2005). Hand washing is recommended as a key method to stay healthy by the Centers for Disease Control, the Association of Practitioners in Infection Control, the Association of Operating Room Nurses, and the US Department of Health and Human Services (Dial Corporation, 2006). Washing properly with the most effective product and method is necessary for optimal bacterial
reduction. People today have hot running water and the benefits of many antimicrobial soaps and hand sanitizers to prevent infections. About 20,000 people die from nosocomial infections each year, and around $500 million would be saved if just 17% of theses nosocomial infections were prevented (Case, 2006). The importance of hand washing should be common knowledge to all and there are countless benefits would we achieve if the simple act of hand washing was practiced properly by everyone in our community.

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Literature Cited


