

A Comparative Study of the Immediate Effects of a Triclosan Antibacterial, Chloroxylenol Antibacterial and Lotion Soap

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ABSTRACT

In response to the University of Wisconsin-La Crosse switching from a liquid soap to a chloroxylenol antibacterial soap, the objective of our research was to perform a comparative study of the immediate effects of a liquid soap, a chloroxylenol antibacterial soap and a more common triclosan antibacterial soap. A group of 29 subjects provided pre-wash and post-wash hand samples utilizing the “glove juice method” and a standardized hand-washing procedure. The collected samples were diluted, inoculated onto Tryptic Soy Agar (TSA) plates and incubated at 35°C for 48 h. The total bacteria grown were quantified by calculating colony forming units (CFUs) from each plate. Specific colony types were then identified using a systematic identification scheme.

After identifying and quantifying the bacteria, the results were compiled and analyzed using a one way ANOVA with repeated measures and a paired t-test. The paired t-test compared the pre-wash and post-wash bacterial counts for the individual soaps and illustrated that each soap reduced a statistically significant amount of bacteria (CFUs). The one way ANOVA compared the three soaps overall and revealed that there was no statistical difference in immediate reduction of bacterial numbers between the tested soaps.

INTRODUCTION

Hands have consistently been an important vehicle in the transmission of disease (1). Thus, thorough hand-washing remains the single most important factor in preventing infection (1). While many studies have focused on the effects of repeated hand-washings in clinical settings, such as that performed before a surgical procedure (3,4); this investigation was designed to compare the immediate effectiveness of three different soaps in a university setting. On a university campus a distinct (non-clinical) environment exists, and the potential value of an antiseptic hand-wash product is based on its ability to reduce bacterial flora following a single application (2).

The soaps compared in this study were antibacterial soaps containing triclosan or chloroxylenol along with a lotion soap that did not contain an active anti-microbial agent. The three soaps were chosen as a model of the University of Wisconsin-La Crosse’s former and current soap, along with another more common triclosan-based antibacterial soap. All of the hand-washing products involved in the study were variations of the institutional Colgate-Palmolive liquid soap line that are available to the University of Wisconsin-La Crosse.

MATERIALS AND METHODS

Subject Selection

Twenty-nine non-clinical volunteers (do not work or come in contact with a clinical or hospital setting) that lacked visible skin injuries, eczema or apparent skin disease were used. During washing and sampling, the wearing of rings and wristwatches was prohibited. The participants were also requested to refrain from using an antibacterial soap eight hours prior to sampling and to refrain from any type of washing of one's hand one hour prior to sampling.

Test Protocol

The study was conducted over a 3 week period in which three soaps, Super Soap Lotion Soap (Colgate Palmolive, Tenaflly, NJ), the triclosan based Super Soap E2 Sanitizing Hand Wash (Colgate Palmolive, Tenaflly, NJ), and the chloroxylenol based Super Soap Antiseptic Soap (Colgate Palmolive, Tenaflly, NJ) were tested. The lotion soap did not contain antimicrobial agents while the chloroxylenol (PCMX) based soap contained 0.5% PCMX. The concentration of triclosan in the E2 Sanitizing Hand Wash was unattainable due to proprietary concerns of the manufacturer. Each week a different soap was tested on all 29 volunteers. At seven day intervals, using a different soap each week, a pre-wash sample (to determine a baseline enumeration of bacteria for that day) and a post-wash sample were obtained.

Pre-wash Sampling

To establish a baseline enumeration of bacteria, a pre-wash sample was collected using the "glove juice method" (3). A sterile non-powdered Nitrile glove (N-Dex, Best Manufacturing Company, Menlo, GA) was aseptically applied to the test subject's hand. Twenty-five ml of sterile Tryptic Soy broth, TSB, (Becton Dickinson, Sparks, MD) was then aseptically added to the gloved hand. The glove was secured to the wrist with self-adherent bandage (Medi-Rip, Conco Medical Company, Rock Hill, SC). The gloved hand was palpated on all surfaces for a period of 1 minute. During this process the subject also clenched a fist twice and dislodged any material from underneath his/her fingernails. The glove juice was then aseptically transferred into a sterile microcentrifuge tube by means of a sterile disposable plastic pipette. Sampling by the glove juice method assisted in the accurate representation of the natural flora on the subject's hands by collecting microorganisms from all surfaces of the hand. Along with the normal flora, this method also recovered potential pathogens residing on the skin.

Hand-washing Procedure

The subject washed his/her hands with a test soap for a regulated two-minute period. With lukewarm tap water running (35 to 37°C), the hands were rinsed and 2 ml of soap was dispensed into the palm. The hands were lathered by rubbing the palms together in a circular motion, followed by the right and left fingers being interlaced and rubbed up and down to create a scrubbing motion between the fingers. Next, the right palm scrubbed the top of the left hand and vice versa. Next, the subject "scratched" the palm of each hand to force the soap under the fingernails. Finally, the hands were rinsed for 1 minute to ensure soap residue left on the hands was minimized. The hands were then dried with paper toweling and the post-wash sample was immediately taken.

Post-wash Sampling

A sterile glove was again donned to the same hand and participant samples were recovered and processed by the same procedure as the pre-wash hand samples. It is to be noted

that preliminary studies demonstrated that sampling the same or opposite hand (relative to pre-wash) generated no difference in the number of bacteria recovered by this method.

Dilution, Plating and Incubation of the Glove Juice Samples

A 1.0 ml sample was taken from the recovered TSB “glove juice” (pre-wash and post-wash) and diluted in sterile TSB. Previous trials established optimal dilutions of 1:10, 1:100, and 1:1000. These dilutions were chosen to attain countable plates. Dilutions were made and 50 µl of each solution was then spread plated onto TSA, (Becton Dickinson, Sparks, MD) plates. All processing of samples was executed within 10 minutes of collection. The plates were incubated at 35°C for 48 h before visual examination.

RESULTS

Bacterial Enumeration and Classification

Bacterial counts were performed on countable plates that consisted of 30-300 bacterial colonies. Each colony was assumed to be one bacterium and thus bacterial numbers were converted to colony forming units (CFUs). To obtain CFUs/ml the number of colonies counted was divided by the dilution of the plate. The total number of bacteria was then enumerated and converted to log₁₀ values. Each unique colony type was also counted and restruct for isolation on TSA plates. The isolated colonies were then characterized using a general classification scheme as illustrated in Figure 1. Because previous studies indicated that catalase positive, Gram positive cocci were the majority of bacteria found on human skin, these isolates were further differentiated into *Staphylococcus* and *Micrococcus*. Moreover, the intent of characterization was not to identify to the species level, but to estimate the prevalence of the different types of organisms found on normal skin (see Figure 2).

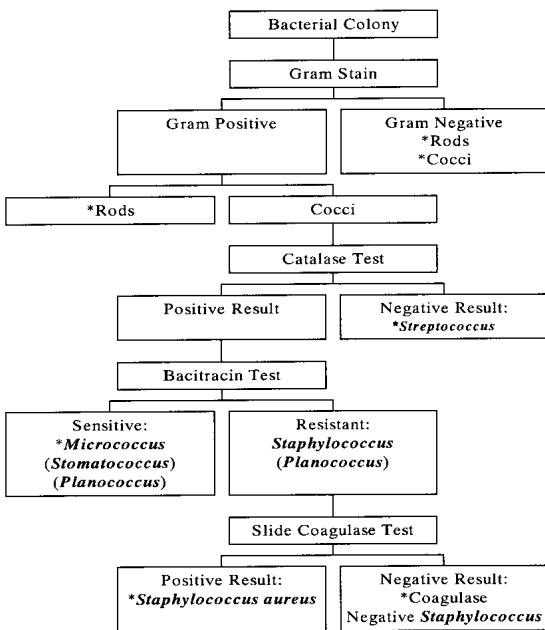


Figure 1. Bacterial classification scheme. (* Denotes reported classifications)

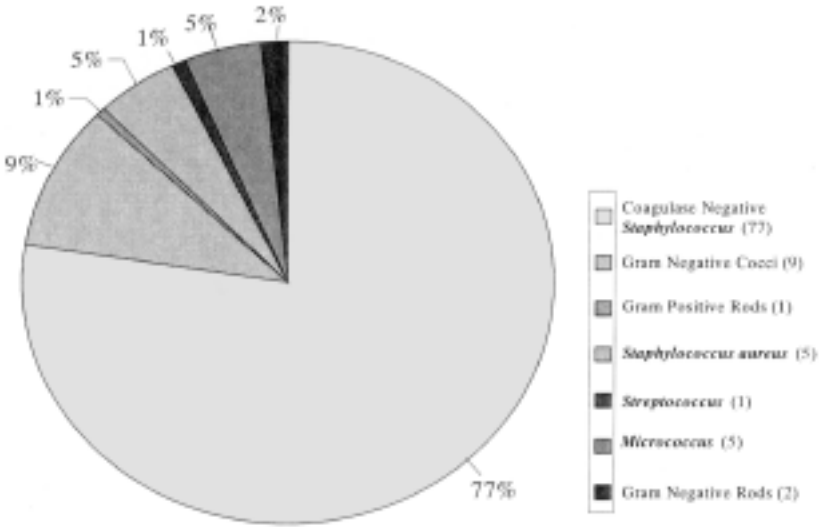


Figure 2. Mean percentage (CFUs/ml) of classified pre-wash isolates.

Statistical Analysis

The mean log₁₀ values of the pre-wash and post-wash samples for each soap were compared by paired t-tests to show whether the immediate reduction for each soap was statistically significant. Using α = 0.05, the samples from all three soaps showed a significant difference between the pre and post-wash (p < 0.001) as seen in Table 1.

Table 1. Pre-wash and post-wash bacterial log₁₀ counts [mean ± standard deviation (n)] as well as difference between pre-wash and post-wash [mean ± standard deviation (n)], 95% confidence interval and P values for each soap product.

	Super Soap Lotion	Super Soap Antiseptic (PCMX)	Super Soap E2 Sanitizing (Triclosan)
Mean ± SD (n)			
Pre-wash	4.87 ± 0.69 (27)	5.20 ± 0.99 (26)	4.97 ± 0.70 (25)
Post-wash	4.43 ± 0.67 (27)	4.78 ± 0.77 (26)	4.49 ± 0.73 (25)
Pre-wash - Post-wash	0.44 ± 0.42 (27)	0.42 ± 0.44 (26)	0.48 ± 0.53 (25)
95% CI	(0.28-0.61)	(0.24-0.60)	(0.26-0.70)
P value	<0.001	<0.001	<0.001

Bacterial reduction for each soap was also calculated in percent reduction (CFUs/ml) along with 95% confidence intervals as seen in Figure 3.

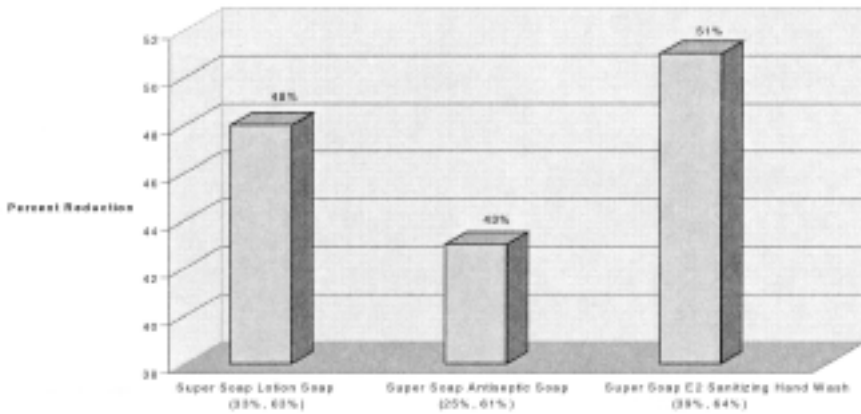


Figure 3. Immediate mean percent reduction (CFUs/ml) and 95% confidence interval for all soap products tested.

A one-way ANOVA with repeated measures was then used to compare the log₁₀ bacterial reduction between all three of the soap products. The ANOVA analysis compared the reduction for each soap in relation to the other soaps. The analysis produced a P value of 0.67 that indicates that there is no statistically significant difference in the bacterial reduction between the soap products.

DISCUSSION

Each of the three soaps provided a significant immediate reduction in the amount of bacteria on the hand following a standardized hand-washing procedure. However, it was also found that there is no difference in the immediate effectiveness between the three soaps. This supports the results of previous research done by Faoagali et al. (3) that there is no immediate difference between a regular lotion soap and an antibacterial soap. In addition, the majority of bacteria found on human hands were coagulase negative *Staphylococcus* (approximately 77%).

While all soaps tested significantly reduced the amount of bacteria on the hands following a hand-wash, all had similar reductions. This may be due to the mechanical action of vigorously rubbing soap and water together for at least 10 seconds which is a major contributor to the cleansing process (9). Washing your hands with an antibacterial soap differs from a plain soap because it leaves a thin residue film of the antibacterial agent on the hands which may retard bacterial growth (8).

Triclosan and PCMX residues have been thought to be nonspecific biocides (3,4). However, recently, studies have shown that triclosan specifically targets fatty acid biosynthesis in bacteria (5,7). Thus, if it targets a specific mechanism, resistant bacterial strains may be selected in its presence. In fact, *E.coli* with a missense mutation in the *fabI* gene have been shown to have a 64-fold higher minimum inhibitory concentration for triclosan when compared to a wild type strain (5). Resistance to triclosan is a significant cause for concern since triclosan is used to combat Methicillin Resistant *Staphylococcus aureus* which can spread in

an environment of immunosuppressed individuals such as that in a hospital. Development of resistance against one antimicrobial (triclosan) is often accompanied by acquisition of resistance to other antimicrobials as well. Such bacterial strains are referred to as being "multi-drug resistant." Dr. Stuart Levy at the Center for Adaptation Genetics and Drug Resistance, as well as other scientists, has noted that we face a growing problem, as more bacteria become multi-drug resistant. Patients infected with these multi-drug resistant strains of bacteria have very limited antibiotic treatment options and face serious health problems(6).

In conclusion, the results of this study (no difference in immediate effectiveness between the three soaps) suggest that the use of an antibacterial soap in the university setting be reconsidered. We must ask what purpose does the antibacterial agent serve in the soap if it does not reduce the bacterial numbers after washing and weigh this against the selection for antibiotic resistant bacterial strains through the widespread use of antibacterial soaps.

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