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Research Paper

Quantifying the Effects of Water Temperature, Soap Volume, Lather Time, and Antimicrobial Soap as Variables in the Removal of *Escherichia coli* ATCC 11229 from Hands

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ABSTRACT

The literature on hand washing, while extensive, often contains conflicting data, and key variables are only superficially studied or not studied at all. Some hand washing recommendations are made without scientific support, and agreement between recommendations is limited. The influence of key variables such as soap volume, lather time, water temperature, and product formulation on hand washing efficacy was investigated in the present study. Baseline conditions were 1 mL of a bland (nonantimicrobial) soap, a 5-s lather time, and 38°C (100°F) water temperature. A nonpathogenic strain of *Escherichia coli* (ATCC 11229) was the challenge microorganism. Twenty volunteers (10 men and 10 women) participated in the study, and each test condition had 20 replicates. An antimicrobial soap formulation (1% chloroxylenol) was not significantly more effective than the bland soap for removing *E. coli* under a variety of test conditions. Overall, the mean reduction was 1.94 log CFU (range, 1.83 to 2.10 log CFU) with the antimicrobial soap and 2.22 log CFU (range, 1.91 to 2.54 log CFU) with the bland soap. Overall, lather time significantly influenced efficacy in one scenario, in which a 0.5-log greater reduction was observed after 20 s with bland soap compared with the baseline wash ($P = 0.020$). Water temperature as high as 38°C (100°F) and as low as 15°C (60°F) did not have a significant effect on the reduction of bacteria during hand washing; however, the energy usage differed between these temperatures. No significant differences were observed in mean log reductions experienced by men and women (both 2.08 log CFU; $P = 0.988$). A large part of the variability in the data was associated with the behaviors of the volunteers. Understanding what behaviors and human factors most influence hand washing may help researchers find techniques to optimize the effectiveness of hand washing.

Key words: Antimicrobial soap; Chloroxylenol; Hand hygiene; Hand washing; Soap volume; Water temperature

The U.S. Food and Drug Administration (FDA) Food Code *(70)* includes recommendations regarding hand washing frequency, duration, and technique; however, the scientific support for many of those recommendations is not always clear nor based on recent evidence. Section 2-301.12 of the Food Code requires the use of a "cleaning compound" (soap) during hand washing. The type of compound is not specified, and facilities may elect to use either bland (soap without an antimicrobial agent) or antimicrobial soap.

Recently, the FDA Center for Drug Evaluation and Research *(71)* issued a final rule establishing that over-the-counter consumer antiseptic washes (soaps) with specific active ingredients may not be marketed in the United States after 6 September 2017. The FDA indicated that the companies that produce these antimicrobial soaps have not provided sufficient evidence to prove that they are safe for daily use and are more effective than bland soap and water. This final rule covers 19 specific active ingredients, including triclosan. However, the FDA has deferred the rule for three ingredients: benzalkonium chloride, benzethonium chloride, and chloroxylenol. This rule does not extend to hand sanitizers or antiseptic wipes and does not address antimicrobial soap sold for use in food service or food processing facilities.

The active ingredients used in antimicrobial soaps disrupt bacterial cell function by either destroying the cell (bactericidal) or inhibiting reproduction (bacteriostatic). These compounds are antiseptics and are not considered antibiotics *(17, 60)*. The literature suggests that antimicrobial soaps provide a greater reduction in bacteria than do bland soaps *(25, 28, 30, 53, 62, 65)*. However, in some studies minimal differences were found *(15, 50, 67)*. A hand soap meta-analysis revealed that use of antimicrobial soaps, when accounting for all types of bacteria and formulations, tended to result in ~0.5-log greater reduction in microorganisms than did use of bland soap *(53)*. Product formulation plays a key role in the effectiveness of antimicrobial agents and soaps, and many active antimicrobial compounds are available for use in soaps, and surfactants in addition to

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other ingredients in soaps or lotions can impede or enhance the activity of these compounds and the overall antimicrobial effect *(14, 26, 69)*.

The combined literature on soap volume (i.e., the dose or amount used per hand washing event) indicates no significant interactions between soap volume and the effectiveness of the soap *(28, 43, 53)*. These data can be confusing and often conflicting when many brands and formulations are compared. Fuls et al. *(28)* found that higher amounts of foaming 0.46% triclosan antimicrobial soap (1.5 to 3 g or two to four pumps of soap) increased the reduction of microorganisms by ~0.7 log units ($P < 0.001$) but did not observe a significant increase in microbial reduction when using a bland soap ($P = 0.2$). Larson et al. *(43)* found that a control wash with bland soap was not significantly affected by the amount of soap used (1 versus 3 mL). However, these researchers also suggested that a higher volume of soap could contribute to skin damage and suggested that the minimal amount of soap required for a thorough wash should be used to reduce the likelihood of skin damage.

The temperature of the wash water required for effective hand washing has not been extensively evaluated and still generates interest. Wash water temperatures have an upper limit; very high temperatures that would rapidly destroy bacterial cells would also severely injure human skin *(42, 68)*. The temperature of the water used during comfortable hand washing would not by itself inactivate resident microbes. Higher temperatures may still affect hand washing by increasing solvation or temperature dependent reaction rates. Boyce and Pittet *(17)* recommended avoiding use of hot water to wash hands because repeated exposure to hot water may increase the risk of dermatitis (damaged skin). Temperatures higher than 55°C can lead to scalding, and the recommended water temperature for human skin comfort is ≤43°C *(42, 68)*. Results of a hand washing survey revealed that hand comfort and personal beliefs played key roles when persons choose the water temperature for hand washing *(19)*. In two studies, Michaels et al. *(49, 50)* found no difference in microbial reductions after hand washing performed at various temperatures (4.4 to 48.9°C). However, the data in these two studies were obtained from only four volunteers, and only one study *(50)* included an antibacterial soap. Courtenay et al. *(21)* measured the differences in microbial reduction between a ServSafe recommended wash (which includes soap), a cool rinse, and a warm rinse. Only minor differences in microbial reduction were found between the cool rinse (26°C) and the warm rinse (40°C), but the interaction between temperature and soap could not be inferred from these data. In a study of various ways to sample bacteria from hands, no significant difference in bacteria recovered was found for sampling solutions at 6 or 23°C *(45)*. Although in all of these studies the temperature of the wash water had no significant antimicrobial effect, the limited replicates *(21, 49, 50)*, comparisons of a wash without soap *(21)*, and lack of actual hand washing *(45)* indicate that more work on the effect of wash water temperature is needed.

The Food Code (section 2-301.12-B-3) *(70)* requires lathering for 10 to 15 s during hand washing. Although specific studies of lather time as a variable have been published, the added friction (from a brush) has been evaluated *(46, 59)* with different results. Price *(59)* found greater microbial reduction with more scrubbing (constant and time dependent), but Loeb et al. *(46)* found no difference in microbial reduction between hand washing with or without a brush. A meta-analysis of the hand washing literature suggested that more studies are needed to understand the importance of wash duration *(53)*. However, many researches who have studied total wash time have suggested that longer wash times are correlated with greater microbial reductions *(25, 28, 34, 47, 55)*. However, results of some studies surprisingly suggest that extended wash times, i.e., >30 s, may result in less effective reduction of transmissible microbes, which would diminish the intended purpose of hand washing *(40, 50, 53)*. One research group hypothesized that extended washing (>30 s) loosens but does not remove resident flora from hands, and these loosened microbes are now more easily transferred to other surfaces, resulting in a reduced overall benefit from removing microorganisms from hands *(50)*. Extended washes and frequent washing can lead to damaged skin *(4, 27, 29, 37–39, 57, 63, 66, 73, 74, 77)*, which promotes colonization by more dangerous microbes and reduces the ability of hand washing to remove bacteria from the (damaged) skin *(40, 42, 44)*. Bidawid et al. *(16)* observed that when finger pads inoculated with hepatitis A virus were rinsed with 15 mL of water, no transfer of virus to lettuce pieces was detected, but when fingers were rinsed with only 1 mL of water, a 0.3% transfer was detected, suggesting that exposure to a greater volume of water may play a key role in hand washing. These conflicting results indicate that more research is needed to determine which hand washing step(s) can be lengthened to increase microbial reduction.

The literature on hand washing includes a tremendous amount of misinformation, and data on many issues are missing. Many hand washing recommendations are being made without scientific backing, and agreement among these recommendations is limited, as indicated by the major inconsistencies among hand washing signs *(35)*. The goal of the present study was to close knowledge gaps in the hand washing literature pertaining to soap volume, water temperature, and lather time. The findings from this work will contribute to valid, evidence-based, helpful decisions concerning personal hygiene policies and practices.

MATERIALS AND METHODS

Volunteers. Twenty-one volunteers were selected from Rutgers University (New Brunswick, NJ) and surrounding communities. Approval from the Rutgers Institutional Review Board was obtained via the standard process before volunteers were enrolled in this study. Volunteers were asked to refrain from using any type of antimicrobial hand soap and non–alcohol-based hand sanitizers for the duration of the study to avoid buildup of active antimicrobial ingredients on the skin, which could have interfered with the results *(2, 12, 28, 54, 56, 64)*. Exclusion criteria included taking antibiotics or being ill during the 6 weeks before the start of the experiment, cuts or abrasions on the hands, self-identification as immunocompromised, or self-identification of discomfort with the experiment and a desire to be removed. One volunteer asked to be removed and did not complete the study. The remaining volunteers (ages 24.5 ± 3.9 years [mean ± SD]) included 10 men (ages 26 ± 2.2 years) and 10 women (ages 23 ± 4.7 years).

Questionnaire. Volunteers were asked to fill out a questionnaire before participation in the experiments. The questionnaire included questions that may account for external variables that could affect skin quality and skin bacterial profiles. The answers were used to parse the volunteers into groups to evaluate whether log reduction data differed significantly between the groups. The demographic variables analyzed were age, sex, moisturizer use, facial cleanser use, medication use, hand washing frequency, recent illnesses, and lotion use.

Experimental design. Four variables (lather time, soap volume, water temperature, and product formulation) were evaluated using a fractional design. One set of conditions (5 s of lather time, 38°C water temperature, and 1 mL of product volume) served as the baseline, and the effect of each variable was studied while holding the other two variables constant. Each unique set of conditions was replicated 20 times such that the total number of experiments was 20 baseline + (3 × 20 lather time) + (2 × 20 water temperature) + (2 × 20 product volume) = 160 hand washes. The entire design was repeated for bland soap and antimicrobial soap containing chloroxylenol, for a total of 320 hand washes. Each volunteer completed 16 hand washes. The target variables to be tested were randomly selected for each experiment. A volunteer performed only one wash per day until there were no more of the 16 sets for a volunteer to perform.

Lather time. Lather times of 5, 10, 20, and 40 s were evaluated. Lather time was defined as the length of time the volunteer lathered soap on their hands (by rubbing hands together) during a hand wash. Lather time did not include initial hand wetting (<1 s), soap application, hand rinsing (held constant at 10 s), or hand drying. Volunteers were instructed to lather their hands in a way that felt most comfortable.

Water temperature. Water temperatures of 38, 26, and 15°C (100, 80, and 60°F, respectively) were evaluated, and the water temperature was verified using a Thermapen with ±0.4°C accuracy (ThermoWorks, Lindon, UT). The temperature of the water was set prior to volunteer arrival and needed to be within ±2°F at the target temperature for at least 60 s. The highest temperature used (38°C) was selected because the FDA Food Code (section 5-202.12) *(70)* indicates that a hand washing sink shall be equipped to provide water at a temperature of at least 38°C. The lowest temperature used (15°C) was deliverable by the existing plumbing and judged by the authors to be the lowest tolerable temperature for comfort.

Estimation of energy consumption. The energy consumption related to heating the water for hand washing was calculated with the following thermodynamic formula:

$$Q = M \cdot C_p \cdot dT/\eta$$

where Q is the amount of heat (kJ); M is mass (kg), representing the amount of water used for a hand wash where a flow of 1 gal (3.8 L) per minute is considered the average water flow with an aerator *(1)* and 10 s is assumed as the rinse time; C_p is the specific heat of water (kJ/kg K) at 4.19; dT is the temperature difference between the heated and ambient water, where an average temperature of 10°C was assumed as the normal temperature for cold tap water and calculations were made for all three temperatures (38, 26, and 15°C); and η is the efficiency of the electric water heater, with an average efficiency of 0.92 based on guidance from the U.S. Office of Energy Efficiency and Renewable Energy *(72)*.

Soap volume. Three volumes of soap were evaluated: 0.5, 1.0, and 2.0 mL. An automatic dispenser (GOJO Industries, Inc., Akron, OH) with a 0.5-mL output was used to dispense the soap. The dispenser was nondescript, had no timer, and did not reveal the formulation being used. This soap dispenser was validated before use each day by catching an aliquot of the foam solution from the dispenser and measuring this aliquot with a scale (Ohaus Scout Pro, Parsippany, NJ). This aliquot was compared with a 0.5-mL volume of the soap that was not converted to foam.

Soap product formulation. Two foaming soap formulations were used for all experiments, one bland soap (i.e., no antimicrobial active ingredients) and one antibacterial soap containing 1.0% chloroxylenol. Both soaps are commercially available (GOJO Industries) and used commonly in a variety of settings, including food service. The soaps were typical in formulation except for the antimicrobial agent and primarily contained a blend of amphoteric and anionic surfactants to remove soils, preservatives, and skin conditioners to soften the skin and balance the effects of the cleansing agents, which can be drying and irritating to the skin. Both soaps were slightly acidic; the pH was 5.2 for the bland soap and 5.5 for the antibacterial soap.

Prewash procedure. Volunteers performed a prewash before beginning the experiment. They were invited into the laboratory and shown the location of the sink but were not given any directions other than to simply wash their hands. No direction was given on how to wash hands or how long to wash. The researcher used a stop watch to discretely measure the amount of soap used, when the hands first touched the water, lather time, rinse time, and total wash time. Volunteers were given paper towels, one at a time, to dry their hands after washing and were given as many towels as requested.

Challenge bacteria. A nonpathogenic strain of *Escherichia coli* (ATCC 11229) served as the challenge bacterium for this experiment. Use of this strain is in accordance with current ASTM International hand washing protocols *(8, 10)*. This strain is a well-established surrogate for transient bacteria transferred to hands during handling of raw foods. Cultures were made followed ASTM method E2946 *(10)*. The *E. coli* was cultured in 10 mL of soybean-casein digest broth for 24 ± 4 h at 35 ± 2°C. This 24-h culture was harvested by centrifugation (Micro 12, Thermo Fisher Scientific, Waltham, MA) at 7,000 × *g* for 10 min and then washed in phosphate-buffered saline (PBS; 0.1 M, pH 7.2). The wash process was repeated three times, and cell pellets were resuspended in PBS to form a challenge suspension of ~8 log CFU/mL.

Hand contamination. One milliliter of the *E. coli* challenge suspension was added to each volunteer's hands. Volunteers were instructed to rub their hands together (10 to 20 s) to cover all surfaces of their hands. Hands were held parallel to the floor to avoid unnecessary contamination of the forearms or elbows. The hands were allowed to dry until they did not appear visibly moist (~40 to 60 s). A sample was collected from the nondominant hand

TABLE 1. *Mean, median, and range of log reductions of microorganisms after various hand washing treatments*

Treatment[a]	Soap formulation	Microbial reduction (log CFU)					
		Mean	SD	Median	Maximum	Minimum	Range
All data	Antimicrobial	1.94	0.78	1.92	4.42	0.06	4.36
	Bland	2.22	0.74	2.22	4.40	−0.04	4.44
Baseline	Antimicrobial	1.92	0.68	1.87	3.13	0.69	2.44
	Bland	1.91	0.64	1.76	2.99	0.82	2.17
Lather time, 10 s	Antimicrobial	2.03	0.64	2.00	3.30	0.89	2.41
	Bland	2.16	0.74	2.22	3.60	1.03	2.58
Lather time, 20 s	Antimicrobial	1.95	1.00	1.82	4.39	0.35	4.03
	Bland	2.54	0.62	2.48	3.75	1.63	2.12
Lather time, 40 s	Antimicrobial	1.91	0.98	2.00	3.47	0.13	3.34
	Bland	2.43	0.71	2.25	4.09	1.57	2.52
Water temp, 15°C	Antimicrobial	1.88	0.62	1.91	3.34	0.76	2.57
	Bland	2.34	0.54	2.33	3.22	1.08	2.15
Water temp, 26°C	Antimicrobial	1.90	0.89	1.77	4.42	0.28	4.14
	Bland	1.98	0.71	1.99	3.07	0.80	2.27
Soap vol, 0.5 mL	Antimicrobial	2.10	0.77	2.18	3.24	0.06	3.18
	Bland	2.25	0.86	2.25	4.03	−0.04	4.07
Soap vol, 2.0 mL	Antimicrobial	1.83	0.65	1.81	3.34	0.64	2.69
	Bland	2.15	0.93	1.97	4.40	0.70	3.70

[a] Baseline treatment was 5-s lather time, 38°C water temperature, and 1-mL soap volume. Other treatments were identical to baseline except as noted. Sample size was 160 for the "all data" category, i.e., $n = 20$ per treatment.

before the hand wash, and that sample was used to calculate the prewash bacterial level.

Bacteria recovery procedure. A modification of the glove juice procedure *(9, 11)* was used to recover bacteria from volunteers' hands. A nitrile glove (powder-free nitrile examination gloves, Thermo Fisher Scientific) filled with 20 mL of PBS was placed over each hand, and the gloved hand was massaged for 60 s to dislodge the bacteria. The glove was then carefully removed, and the rinsate was poured into a collection tube (Falcon 50 mL Conical Centrifuge Tubes, Corning, Inc., Corning, NY). Tween 80 (10%) was used as a neutralizer in the sampling buffers for the antimicrobial soap experiments *(7)*. Neutralization of the antimicrobial agent was confirmed using ASTM method E1054-08, section 9 (neutralization assay with recovery in liquid medium) *(6)*.

Sample dilution and plating. PBS (pH 7.2 ± 0.1) was used for serial dilutions and contained the neutralizer when necessary. Samples were plated onto MacConkey agar (BBL, BD, Sparks, MD), and the CFUs were enumerated after incubating for 24 h at 35°C. The medium contained 4-methylumbelliferyl-β-D-glucuronide (Sigma-Aldrich, St. Louis, MO) to allow identification of *E. coli* without affecting colony morphology or viability *(52)*.

Hand washing. Volunteer hand washing experiments were focused on the four variables: lather time, water temperature, soap volume, and soap formulation. Volunteers were given additional instructions as to how much soap to use (number of pumps), when to wet their hands, when to stop lathering, and when to stop rinsing. Volunteers were not told what formulation they were using or the water temperature. Volunteers did not dry their hands to avoid removal of bacteria with the paper towel *(20, 32–34, 75)*.

Postwash sampling. Samples were collected from volunteers' hands immediately after the wash (<5 s). Both hands were sampled using the modified glove juice method *(9, 11),* and these samples were used to calculate the postwash bacterial levels.

Postexperiment decontamination protocol. Before leaving the testing area, volunteers washed their hands under running water for 20 s using bland soap and dried their hands with paper towels. One pump of alcohol-based hand sanitizer (Purell, GOJO Industries) was then applied to the volunteers' hands, and volunteers were asked to rub their hands together until the sanitizer was completely dry. The volunteers were then asked to leave the testing area.

Data analysis. Microbial reduction data gathered from the experiment were log transformed to achieve a normal distribution *(61)*. The log reduction was determined by taking the logarithm of the prewash bacterial level on the nondominant hand (multiplied by 2 to estimate the level on both hands) and subtracting from that the logarithm of the sum of the postwash level on both hands.

A repeated-measures analysis of variance (ANOVA) and Tukey's range test and honest significant difference (HSD) test (Prism, GraphPad Software, La Jolla, CA) were used to determine whether multiple means were significantly different and whether any significant interactions existed between the variables. Differences were considered significant at $P < 0.05$. For scenarios in which only two variables were being compared, including when comparing groups from the questionnaires, a two-tailed *t* test was used to calculate *P* values (Excel, Microsoft, Redmond, WA) to determine whether significant differences existed between samples.

RESULTS

Table 1 shows the overall log reductions for all treatment conditions tested and the mean log reductions overall for the antimicrobial soap containing chloroxylenol and the bland soap. Overall, the antimicrobial soap produced a mean (SD) 1.94 (0.78)-log CFU reduction in microbial levels (range, 1.83 to 2.10 log CFU). The bland soap produced a mean (SD) 2.22 (0.74)-log CFU reduction

TABLE 2. *ANOVA of scenarios and volunteers*

Variable	Soap formulation	SD	Degrees of freedom	Mean square
Between volunteers	Antimicrobial	0.9985	7	0.1426
	Bland	6.465	7	0.9235
Between scenarios	Antimicrobial	27.37	19	1.441
	Bland	26.2	19	1.379
Residual	Antimicrobial	68.08	133	0.5119
	Bland	54.5	133	0.4098
Total	Antimicrobial	96.45	159	
	Bland	87.17	159	

(range, 1.91 to 2.54 log CFU). The analysis revealed a significant effect for soap formulation ($P = 0.00025$).

An ANOVA was performed to observe differences within the data sets and between volunteers (Table 2). The analysis revealed a significant difference between volunteers ($P < 0.0001$) (person-to-person variability factors). The post hoc Tukey HSD test on the individual volunteer's mean log reduction data revealed significant differences ($P < 0.05$, data not shown). Multiple mean log reduction differences $\geq$0.5 log CFU were found between the volunteers, which suggests that a large part of the variability in the data sets were due to variability between the volunteers. A subsequent Tukey HSD test was performed to determine differences between the individual scenarios (Table 3) to make sure that differences between scenarios were not overlooked when the two groups were combined. The analysis included lather time, water temperature, and soap volume as independent variables; the data were separated by soap formulation. For the bland soap, significant differences were found for lather time ($P = 0.01$). A post hoc HSD test revealed that the bacterial reductions with the 20-s lather time were significantly different from those achieved with the baseline lather time of 5 s ($P = 0.01$) but were significantly different from reductions achieved with the 10- and 40-s lather times. For bland soap, no significant effects on bacterial reduction were found for soap volume ($P = 0.23$) and water temperature ($P = 0.08$). For the antimicrobial soap, no significant effects on bacterial reduction were found for lather time ($P = 0.85$), water temperature ($P = 0.97$), and soap volume ($P = 0.22$). However, for the antimicrobial soap data, the P values were higher for lather time and water temperature (lather time, $P = 0.85$; temperature, $P = 0.97$) than for the bland soap data (lather time, $P = 0.01$; temperature, $P = 0.08$).

Higher water temperature entails greater energy consumption (see Fig. 1). The energy consumption associated with heating water for 1,000 hand washes is 22.35 kWh for a water temperature of 38°C but only 12.77 kWh for a water temperature of 26°C, which is a reduction of 42%. The

TABLE 3. *Tukey multiple comparison test results for antimicrobial and bland soap*

Comparison	Antimicrobial			Bland[a]		
	Mean difference	q	95% CI	Mean difference	q	95% CI
Baseline vs lather 10 s	−0.110	0.687	−0.8079 to 0.5880	−0.244	1.708	−0.8689 to 0.3800
Baseline vs lather 20 s	−0.030	0.188	−0.7280 to 0.6679	−0.628*	4.384*	−1.252 to −0.003004*
Baseline vs lather 40 s	0.010	0.064	−0.6877 to 0.7082	−0.521	3.641	−1.146 to 0.1034
Baseline vs temp 15°C	0.033	0.207	−0.6648 to 0.7311	−0.427	2.982	−1.051 to 0.1977
Baseline vs temp 26°C	0.011	0.072	−0.6865 to 0.7094	−0.071	0.497	−0.6956 to 0.5533
Baseline vs vol 0.5 mL	−0.182	1.134	−0.8794 to 0.5165	−0.339	2.369	−0.9635 to 0.2854
Baseline vs vol 2 mL	0.083	0.518	−0.6151 to 0.7808	−0.233	1.625	−0.8571 to 0.3918
Lather 10 s vs lather 20 s	0.080	0.500	−0.6180 to 0.7779	−0.383	2.676	−1.008 to 0.2414
Lather 10 s vs lather 40 s	0.120	0.752	−0.5777 to 0.8182	−0.277	1.933	−0.9012 to 0.3478
Lather 10 s vs temp 15°C	0.143	0.895	−0.5548 to 0.8411	−0.182	1.274	−0.8068 to 0.4421
Lather 10 s vs temp 26°C	0.122	0.759	−0.5765 to 0.8194	0.173	1.211	−0.4512 to 0.7977
Lather 10 s vs vol 0.5 mL	−0.072	0.447	−0.7695 to 0.6265	−0.095	0.661	−0.7191 to 0.5299
Lather 10 s vs vol 2 mL	0.193	1.205	−0.5051 to 0.8908	0.012	0.082	−0.6127 to 0.6363
Lather 20 s vs lather 40 s	0.040	0.252	−0.6576 to 0.7383	0.106	0.743	−0.5181 to 0.7308
Lather 20 s vs temp 15°C	0.063	0.395	−0.6347 to 0.7612	0.201	1.402	−0.4238 to 0.8252
Lather 20 s vs temp 26°C	0.042	0.260	−0.6564 to 0.7395	0.556	3.887	−0.06816 to 1.181
Lather 20 s vs vol 0.5 mL	−0.151	0.947	−0.8494 to 0.5465	0.288	2.015	−0.3360 to 0.9129
Lather 20 s vs vol 2 mL	0.113	0.706	−0.5850 to 0.8109	0.395	2.758	−0.2296 to 1.019
Lather 40 s vs temp 15°C	0.023	0.143	−0.6751 to 0.7209	0.094	0.659	−0.5301 to 0.7188
Lather 40 s vs temp 26°C	0.001	0.008	−0.6967 to 0.6992	0.450	3.143	−0.1745 to 1.074
Lather 40 s vs vol 0.5 mL	−0.192	1.199	−0.8897 to 0.5062	0.182	1.272	−0.4424 to 0.8065
Lather 40 s vs vol 2 mL	0.073	0.454	−0.6253 to 0.7706	0.289	2.015	−0.3360 to 0.9129
Temp 15°C vs temp 26°C	−0.022	0.136	−0.7196 to 0.6763	0.356	2.484	−0.2688 to 0.9801
Temp 15°C vs vol 0.5 mL	−0.215	1.342	−0.9126 to 0.4833	0.088	0.613	−0.5367 to 0.7122
Temp 15°C vs vol 2 mL	0.050	0.311	−0.6482 to 0.7477	0.194	1.356	−0.4303 to 0.8186
Temp 26°C vs vol 0.5 mL	−0.193	1.206	−0.8909 to 0.5050	−0.268	1.872	−0.8924 to 0.3566
Temp 26°C vs vol 2 mL	0.071	0.446	−0.6266 to 0.7694	−0.162	1.128	−0.7860 to 0.4630
Vol 0.5 mL vs vol 2 mL	0.264	1.652	−0.4336 to 0.9623	0.106	0.743	−0.5181 to 0.7309

[a] * $P < 0.05$.

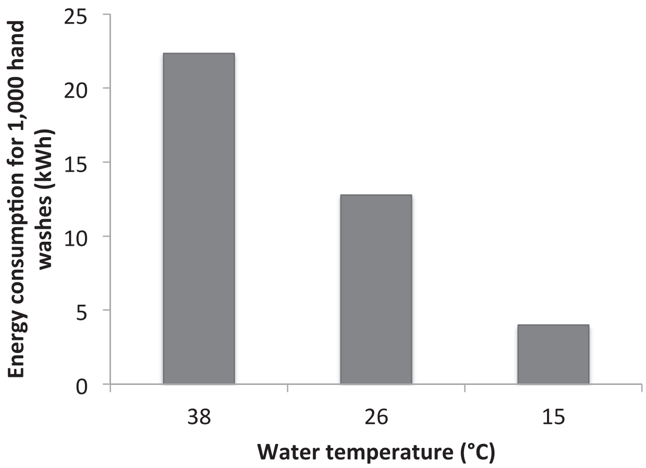

FIGURE 1. *Energy consumption related to water heating for hand washing.*

energy consumption associated with heating water for 1,000 hand washes is only 3.99 kWh for a water temperature of 15°C, which is a reduction of 68% compared with the baseline of 38°C.

Questionnaire results. No significant differences in bacterial reductions were found for volunteers who did versus did not use acne medication ($P = 0.14$) or facial cleanser ($P = 0.62$). Volunteer age also did not have an effect on mean log reductions ($r^2 = 0.009$, $P = 0.09$).

Lotion use. The questionnaire results indicate a significant difference in mean log microbial reduction ($P = 0.02$) for volunteers based on high use of lotion (2.15 log CFU) versus low use of lotion (1.95 log CFU). The difference between volunteers who used lotion and those who did not use lotion was ~0.2 log CFU.

Hand washing frequency. Sixteen volunteers indicated that they typically washed their hands more than four times per day, and four volunteers indicated that they washed their hands fewer than four times per day. The prewash mean total wash time differed significantly between these two groups ($P = 0.012$); the high frequency hand washers washed for an average of 18.2 s, and the low frequency hand washers washed for an average of 15 s. Further analysis revealed that the difference in wash times was due to lather time, not rinse time. No significant difference was found for mean rinse times ($P = 0.714$), but a highly significant difference in mean lather time was found ($P = 0.000022$); frequent hand washers lathered for 6.8 s, and less frequent hand washers lathered for 4.0 s. Washing was significantly more effective for the low frequency hand washers than for the high frequency hand washers ($P = 0.0008$) with an mean log reduction of 2.37 log CFU for low frequency washers and 2.01 log CFU for high frequency washers. This difference was still significant when accounting for formulation (antimicrobial soap, $P = 0.048$; bland soap, $P = 0.0045$). The four low frequency hand washers also reported the highest usage of lotion (more than twice per day), which improved hand washing efficacy.

Men versus women. No significant difference in mean log reductions was found for men (2.08 log CFU) and women (2.08 log CFU) ($P = 0.988$). The P value did not change for the antimicrobial or bland soap. However, a significant improvement in mean log reduction (2.34 log CFU) was found for men who used lotion versus men who did not use lotion (1.90 log CFU) ($P = 0.0003.9$). This same comparison for women was not possible because all of the women volunteers reported using lotion at least once per day (high lotion usage).

Prewash data. Breakdown of the prewash data is shown in Table 4. During the prewash phase, the mean recorded lather time was 6.3 s, the mean rinse time was 11.4 s, and the mean total wash time was 17.7 s. The temperature of the wash water did not change the observed lather ($P = 0.76$), rinse ($P = 0.31$), and overall wash ($P = 0.70$) times. For both men and women, no effect of water temperature on the observed wash times was found, and the respective P values remained roughly the same. Men lathered and rinsed their hands for a longer time (~2 s) than did women (lather time: men = 7.4 s, women = 5.4 s, $P = 0.006$; rinse time: men = 12.3 s, women = 10.5 s, $P = 0.04$), which resulted in a longer overall hand washing times for men ($P = 0.002$). Minimal correlation was found between length of lather time and rinse time ($R^2 = 0.03$) for all volunteers. The mean (SD) volume of soap used was 0.6 (0.25) mL (Fig. 2; approximately one pump of soap) for both men and women. Although the difference between men and women for volume of soap used was not significant ($P = 0.39$), further analysis revealed a significant difference in volume of soap used across all volunteers ($P = 0.000000135$), suggesting that personal behavior dictated choice of soap volume; 71% of volunteers used one pump, 26% used two pumps, 1% used three pumps, and 2% used no pumps of soap. These percentage differences did not noticeably change with water temperature. A volunteer did not change the number of pumps of soap used for each prewash and would routinely use the same amount of soap. A weak correlation (low R^2) was found between total wash time and pumps of soap used ($P = 0.001$, $R^2 = 0.07$), and 43.4% of volunteers used water before applying soap, whereas 56.6% applied soap before using water. For the men, 56.8% used water first and 43.2% used soap first; for the women, 31.1% used water first and 68.9% used soap first.

DISCUSSION

Lather time (length of wash). The 30-s wash (20 s of lathering and 10 s of rinsing) with bland soap produced a significantly different mean log reduction in bacterial counts compared with the baseline 15-s wash. Results of several other studies have indicated that a longer wash time can provide a greater microbial reduction benefit *(25, 28, 34, 47, 55)*. However, these studies involved an overall wash time of <30 s and did not break the wash event into separate parts (lather versus rinse). In a meta-analysis of hand

TABLE 4. *Prewash data*[a]

Group	Total no. of washes	Mean wash time (s)			% volunteers using:					
		Lather	Rinse	Total	No soap	One soap pump	Two soap pumps	Three soap pumps	Water first	Soap first
All	198	6.3	11.4	17.7	2.0	70.7	26.3	1.0	43.4	56.6
15°C	31	7.0	10.6	17.6	0.5	11.1	4.0	0.0	6.6	9.1
26°C	47	6.1	12.5	18.6	0.5	16.7	6.1	0.5	9.1	14.7
38°C	120	6.3	11.1	17.4	1.0	42.9	16.2	0.5	27.8	32.8
Men	95	7.4	12.3	19.7	3.0	62.0	29.0	1.0	56.8	43.2
15°C	19	7.6	11.4	19.0	1.0	12.0	6.0	0.0	11.6	8.4
26°C	20	6.2	13.3	19.5	1.0	14.0	5.0	0.0	11.6	9.5
38°C	56	7.8	12.2	19.9	1.0	36.0	18.0	1.0	33.7	25.3
Women	103	5.4	10.5	15.9	1.0	78.0	23.0	1.0	31.1	68.9
15°C	12	6.0	9.3	15.3	0.0	10.0	2.0	0.0	1.9	9.7
26°C	27	6.3	11.9	18.0	0.0	19.0	7.0	1.0	6.8	19.4
38°C	64	4.9	10.2	15.1	1.0	49.0	14.0	0.0	22.3	39.8

[a] Percentages are of 198 washes for the "all" group, 95 washes for the men, and 103 washes for the women. Some of the prewash data were compromised (equipment malfunction), resulting in a different number of prewashes for men and women. Each pump of soap provided 0.5 mL of foaming product.

washing, 120-s washes resulted in a lower log reduction than did 30-s washes *(53),* suggesting that wash times >30 s may not be more effective. These results are consistent with our findings and suggest that microbial reduction will not increase significantly beyond 10- to 20-s lather times. One hypothesis to explain this finding is that microbes that are easier to remove are lifted from the hands by the wash in <30 s; however, microbes that are embedded in deeper layers or pores or are biochemically attached to skin will not be removed regardless of longer hand washing time.

Water temperature. In our study, no significant difference in washing effectiveness was found at different temperatures (15 to 38°C). This finding agrees with those of Michaels et al. *(49, 50),* who tested a wider range of water temperatures (4.4 to 48.9°C) but found mean microbial reductions of ∼2 to 2.5 log CFU, very similar to our mean reductions of 1.9 to 2.3 log CFU. Courtenay et al. *(21)* found a small but significant difference (94 versus 99%; $P < 0.05$)

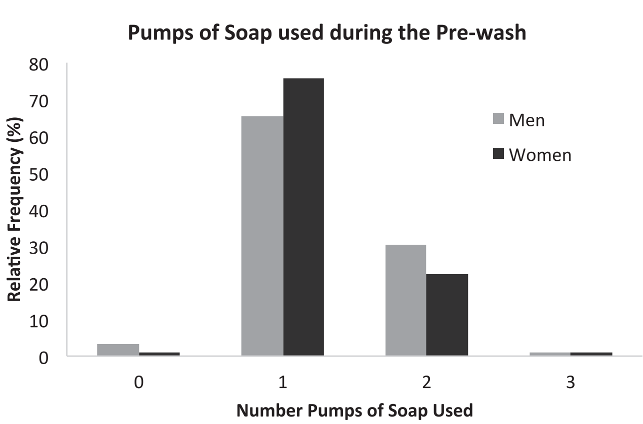

FIGURE 2. *Number of pumps of soap used by women (solid) and men (shaded) during the prewash. Each pump delivered 0.5 mL of soap.*

in microbial reduction between a cool rinse (26°C) and a warm rinse (40°C), but because none of these experimental washes included the use of soap, the relevance to a hand washing following the recommendation of the FDA Food Code *(70)* is unclear. Because Courtenay et al. studied hands inoculated with a ground beef matrix, the saturated fats in the meat may have been more easily removed at warmer water temperatures. Warmer water does not enhance antimicrobial activity but have a negative environmental impact (i.e., energy consumption); therefore, policy requirements for warm water hand washing (e.g., the Food Code) should be reconsidered.

Volume of soap. No significant difference for volume of soap used was found for either kind of soap (bland soap, $P = 0.48$; antimicrobial soap, $P = 0.41$). Both Fuls et al. *(28)* and Larson et al. *(43)* found no significant increase in microbial reduction when using bland soap. However, in contrast to our findings, Fuls et al. and Larson et al. did find that increasing the volume of the antimicrobial soap increased the log reductions. Both sets of authors suggested increased exposure to more antimicrobial agent as the explanation for increased microbial reduction. The difference in mean log reductions for a higher volume of antimicrobial soap may be due to the types of active agents being tested because formulation effects efficacy *(14, 69).* We used a 1% chloroxylenol antimicrobial soap, Larson et al. used a 4% chlorhexidine gluconate antimicrobial soap, and Fuls et al. used a 0.46% triclosan antimicrobial soap. The minimum volume of soap needed should also consider the soil removal required by the users, which is also likely to be significantly affected by soap formulation (especially surfactant choices).

Antibacterial and bland soaps. A significant difference in microbial reduction was found between soap formulations ($P = 0.0003$). However, the difference in mean log reductions between the antimicrobial and bland soap (Table 1) was only ~0.3 log CFU, which is within the range of error for microbiology data (i.e., a clinically insignificant difference). In several studies, greater microbial reductions were achieved with antimicrobial soaps than with bland soaps *(25, 28, 30, 62, 65),* and the effectiveness of antimicrobial soaps increased with repeated use by building up the antimicrobial agent on the skin *(2, 12, 28, 54, 56).* This effect can also be seen with hand sanitizers made with antimicrobial agents that remain on the skin *(64),* unlike those made with alcohol, which is not readily absorbed *(13, 18).* Given the FDA 1-year extension for soaps containing chloroxylenol *(71),* future work with the antimicrobial soap used in this study should take into consideration the need for buildup on the skin to improve efficacy and formulation style. In their meta-analysis of hand soaps, Montville and Schaffner *(53)* suggested that overall, accounting for all types of bacteria, antimicrobial soap should have a ~0.5-log greater reduction (mean, 2.4 log CFU) than bland soap (mean, 1.9 log CFU). We did not see a greater difference, but the bland soap data and the antimicrobial soap data both fell within the meta-analysis's range of mean log reductions *(53).* Future studies should take into consideration the surfactant profile of an antimicrobial soap, which can have a significant effect on the results *(14, 69).* We used two formulations that were both commonly used by the public and designed to be mild to the skin and similar in use. Highly efficacious antimicrobial soaps are made by designing the ingredient matrix around the antimicrobial active ingredient to create a formulation that does not inhibit but ideally highly activates the antimicrobial agent *(14, 69).* Future work should take into consideration the variety of antimicrobial soaps available and the various methods for testing these soaps.

Lotion use. Although the mean differences were small (~0.2 log CFU) between lotion users and non–lotion users, lotion use could affect several analyses. Skin damage from frequent hand washing is a well-established phenomenon *(4, 27, 29, 37–39, 57, 63, 66, 73, 74, 77),* and lotion often is used to repair this damaged skin *(5, 41, 48).* Damaged skin is more difficult to wash *(40, 42, 44),* so a slight, yet higher log reduction for the volunteers who indicated regular lotion use is not surprising. Although all women indicated using lotion more than once per day, not all men used lotion regularly (~0.5 log CFU greater mean reduction for men who were lotion users). This study did not provide sufficient evidence to draw a strong conclusion about the effect of lotion use on hand washing. However, the available evidence is enough to warrant more precisely controlled and designed investigations to measure the effect of hand lotion use on hand washing. Use of lotion to improve skin quality *(5, 41, 48)* and reduce pathogen colonization of damaged skin *(40, 42, 44)* would be an advantage to both health care workers and food handlers.

Person-to-person variability. A large part of the variability in the data sets was due to variability between the volunteers (Table 2). This finding is not uncommon for in vivo hand washing research, and large variability in results can be found both within and between hand washing studies *(53).* Microbial reductions >4 log CFU have been consistently reported in hand sanitizer research, with limited variability *(3, 22–24, 31, 36, 51, 58, 76),* suggesting that hand soap and hand sanitizer effectiveness may be more influenced by human behavior and/or physiological hand differences than by the effectiveness of the soap and/or sanitizer, which is not surprising considering the number of steps recommended for proper hand washing *(35).* No published work was discovered that links physiological differences, such as skin moisture levels, skin sensitivity, hair density, scar tissue, and hand size, to hand washing outcomes. How these physiological differences affect microbial loads, reductions, and health risks would be an interesting topic for future hand hygiene research.

Other observations. Similar to our work, Larson et al. *(43)* also recorded the mean amount of soap (mL) used by health care workers. They observed that health care workers used ~ 2.7 mL of soap when attending to high-risk patients, ~2 mL when attending to low-risk patients, and ~1 mL when not attending to patients. Our volunteers, who were not health care workers, used a much smaller amount of soap than did the participants in the study by Larson et al. (mean, 0.6 mL for the prewash; Fig. 2); 65% of men used one pump of soap, and 75% of women used one pump of soap. Larson et al. did not use a foaming soap but rather a liquid soap in a syringe dispenser and asked the volunteers to use an amount of soap they would normally use for hand washing. In our study, soap was released in 0.5-mL increments from a dispenser. Similar to the Larson et al. study *(43),* we found that volunteers used different amounts of soap, and each volunteer routinely used the same amount of soap for each of hand wash, i.e., consistently following their individual habits.

The results of this study indicate that water temperature is not a critical factor for the removal of transient microorganisms from hands. Combining these results with those of other studies of water temperature as a variable *(49, 50),* water temperature does not have a strong effect on hand washing. Therefore, it may be time to remove water temperature recommendations for hand washing from regulations and promote recommendations aimed at skin comfort *(42, 68).* Overall, the length of lather time and volume of soap used did not make a large difference, but a minimum of 0.5 mL of soap and 10 s of lather time is recommended based on our findings. Lotion use by the volunteers had an effect on the results; microbial reduction was greater for volunteers that used lotion regularly. One of the key findings from this study is that variability exists between people in both microbial reduction after hand washing and hand washing behavior. Understanding which behaviors, human factors, and physiological differences influence hand washing the most may allow future studies to focus on which techniques can optimize the effectiveness of hand washing and thereby reduce infection transmission risk and improve food safety.

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