Water temperature as a factor in handwashing efficacy

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Abstract

For many years, sanitarians have specified that the hands of food service workers should be washed and rinsed in warm or hot water to reduce the risk of cross-contamination and disease transmission. In the food service environment, it has been suggested that handwashing with water at higher temperatures contributes to skin damage when frequent handwashing is necessitated, and that insistence on hot water usage is a deterrent to handwashing compliance. Separate handwashing studies involving different water temperatures and soap types (antibacterial versus non-antibacterial) were performed. The 'glove-juice' technique was employed for microbial recovery from hands in both studies. Initial work evaluated antimicrobial efficacy based on water temperature during normal handwashing with bland soap. Uninoculated, sterile menstrua (tryptic soy broth or hamburger meat) was used to study the effects of treatment temperatures (4.4°C, 12.8°C, 21.1°C, 35°C or 48.9°C) on the reduction of resident microflora, while Serratia marcescens-inoculated menstrua was used to evaluate treatment effects on the reduction of transient contamination. Results of this first study indicated that water temperature exhibits no effect on transient or resident bacterial reduction during normal handwashing with bland soap. The follow-up study examined the efficacy and skin irritation potential involving water temperatures with antimicrobial soaps. Hands of participants were contaminated with *Escherichia coli* inoculated ground beef, washed at one of two water temperatures (29°C or 43°C) using one of four highly active (USDA E2 equivalency) antibacterial soaps having different active ingredients (PCMX, Iodophor, Quat or Triclosan). Skin condition was recorded visually and with specialized instrumentation before and after repeated washing (12 times daily), measuring total moisture content, transepidermal water loss and erythema. Overall, the four soap products produced similar efficacy results. Although there were slight increases in Log_{10} reductions, visual skin irritation, loss of skin moisture content and transepidermal water loss at higher temperatures, results were not statistically significant for any parameter.

Introduction

A critical and thorough evaluation of simple handwashing procedures reveals numerous variables to be considered by food service managers in order to achieve maximum or appropriate de-germing of the hands and fingernail regions. Numerous studies have explored issues such as type of soap (i.e. antibacterial versus plain, liquid versus bar), amount of soap, nailbrush use, drying technique (i.e. cloth versus paper towels, paper towels versus air-drying), and application of instant hand sanitizers (postwash liquids). Previous studies indicate that these variables are crucial in achieving effective removal of transient bacteria from the hands under controlled testing conditions. Rarely mentioned in the scientific literature is testing to determine specific guidelines for water temperatures and flow rates. Many of the currently employed hand-

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washing practices are based on untested traditions that could possibly result in compromised skin health. It is expected that warm or hot water would be beneficial in reducing bacterial counts from hands during handwashing, as heat provides energy for the increased solubility and melting of fats, oils and other soils which may serve as vehicles for bacterial transfer from hands. Warm/hot water, combined with the detergents present in soap, should theoretically provide greater emulsification of contaminating soils on the skin, resulting in a more efficient lifting of these soils for rinsing away.

Some food safety experts strongly recommend the use of antimicrobial soaps for food service workers, while others are now focusing on handwashing frequency. With the rise of antibiotic resistance, increased concern has been expressed with respect to antimicrobial soap usage. The reasoning has been that when warm/hot water is combined with antimicrobial soap, the temperature of activation is approached, accelerating chemical reactions and improving kill rates. Soil emulsification should allow for greater exposure of microorganisms in the contaminating soil to the antimicrobial active agents. Thus, bacterial population numbers may be reduced two ways: through soil emulsification and lifting/rinsing away, and inactivation provided by the antimicrobial agent(s) with higher temperatures doing a significantly better job. The infected food worker is the focus of improved hygiene measures, and food safety managers and regulators would be remiss to not try to optimize effectiveness. Asymptomatic food handlers have been identified as being responsible for approximately one-third of outbreaks traced back to the infected worker. Poor personal hygiene has been cited as a contributory factor in an average of 30% of foodborne illness outbreaks occurring in the U.S. between the years of 1973 and 1997 (Bean & Griffin 1990; Bean *et al*. 1996; Olsen *et al*. 2000). The vast majority of foodborne illness outbreak cases attributed to the infected food handler occurs in the food service environment (Michaels *et al*. 2002).

The main initiative in hand hygiene is the reduction of potentially pathogenic microorganisms from contaminated skin surfaces. Optimization of all variables involved in this task must not only provide sufficient removal and/or kill of potential pathogens, but must also refrain from damaging the skin, as this can affect handwashing compliance (Boyce and Pittet 2001) and seriously compromise food service safety. Skin damage associated with work from routine and frequent handwashing has also been seen to result in colonization of workers hands with potential pathogens.

With so many variables involved in such a 'simple procedure', it would make sense to explore and maximize all possible aspects of the process while minimizing negative collateral. This is especially important due to the many observations of food service workers revealing what is considered to be poor habits in handwashing techniques. Studies indicate that handwashing compliance drops considerably without supervision and monitoring, or in situations where skin damage occurs. This further amplifies the need to strengthen knowledge of all variables that might improve or weaken daily handwashing practices throughout the food processing and service industry.

As described by Price, two types of flora exist on the hands, transient and resident species (Price 1938). The transient flora is generally removed fairly easy. They do not have adhesion characteristics that hold them to the skins' surface and are somewhat suppressed by secretions and competitive exclusion by the resident flora (Dunsmore 1972). Resident flora is removed more slowly. Because of coevolution, resident flora have adapted to conditions on the skins' surface that cause rapid die-off of most transients. Invaginations such as the nail fold, hair follicles and sebum-producing sebaceous glands support a rich resident flora. Transient flora may consist of pathogens, spoilage bacteria or harmless environmental species. Under certain conditions, transient flora can change status and become permanent residents. Resident flora, as a rule, are not pathogenic types. Although colonization with coagulase-positive staphylococcus is fairly common (Noble & Pitcher 1978). Frequent or prolonged exposure of the skin to microbial contamination in soils, skin damage or fissures provide portals of entry to deeper tissue, and may result in many pathogenic bacteria found among the resident species (Price 1938; Kaul & Jewett 1981). Food workers in a number of different food industry segments (including catering and bakery) have been found colonized by varying numbers of potential pathogens (Seligman & Rosenbluth 1975).

The effective water temperature used for washing and rinsing hands was a topic of intense discussion at the U.S. Year 2000 Conference for Food Protection. This biannual conference assembles federal and state regulators, food safety academicians, food service industry scientists and safety managers to establish and recommend guidelines to the United States Food and Drug Administration (FDA) for inclusion into the FDA Model Food Code. This code, as adopted by individual US states, forms the basis for food safety regulation and enforcement activities to the food service industry. Several submitters of issues, brought before science and technology council (Council III), expressed their concern regarding the use of higher water temperatures as recommended of the food service/processing industry (Table 1). The United States Food and Drug

Table 1 Submitters and handwashing water temperature issues at the year 2000 Conference for Food Protection

Submitter	Issue	Reason
L. Wisniewski (Select Concepts – Consulting)	'Warm Water'	1. Hand Discomfort Decreases Frequency
M. Scarborough (Georgia Department of Human Resources, Division of Public Health)	37.7°C (100°F)	1. No Science (43°C vs. 37.8°C) 2. Plumbing Code @ 100°F Max. (Safety Concerns)
J. Budd (Healthminder/Sloan Valve Company)	35°C (95°F)	1. No Scientific Basis 2. Max Soap Efficacy at 35°C 3. Hand Comfort 4. Hot Water Discourages Hand Washing
E. Rabotoski (Wisconson ConferenceFood Protection)	'Tempered' 29.5°C (85°F) to 43°C (110°F)	1. Hand Discomfort 2. Possible Scalding
B. Adler (Minnesota Department of Health)	Impose Temp. Range 43°C 110°F To 54.4°C (130°F)	1. Need upper limit or subject to OSHA 2. Food workers Don't Wash 25 Sec. So Cannot Scald.
Reimers (H.E.B. Grocery Company)	'Tempered' To Warm	1. No Science . 2. Max Soap Efficacy 3. 43°C Risks Injury 4. Waste Water as Wait for Temp. at 43°C

Administration (FDA) Food Code provides recommendations for the food service industry to follow regarding food handling practices, application of HACCP principles and personal hygiene implementation (US Public Health Service 1999; US Public Health Service 2001). The main goal of the FDA has been the creation of uniform practices throughout all of the United States. The 1999 FDA Food Code requires sinks used for handwashing to be equipped so as to be 'capable of providing water of at least 43°C (110°F), accomplished through use of a mixing valve or a combination faucet' [tap] (US Public Health Service 1999).

All but one of the submitters requested temperature decreases with the intent of improving hand comfort, as the discomfort associated with higher temperatures results in decreases in hand washing frequency or compliance. Several submitters note a lack of scientific information on the subject. There is concern that a minimum handwashing temperature of 43°C (110°F), in addition to causing discomfort, will result in injury or scalding and may even be in conflict with local plumbing codes. Two submitters point out that soaps currently available target maximum effectiveness at around 35°C (95°F). Two submitters requested that the minimum temperature of 110°F (43°C) be changed to warm water or that it be tempered to a range of 85°F (29.5°C) to 110°F (43°C). and finally, one submission sought to place an upper temperature limit of 130°F (54.4°C), for fear that these regulations would be subject to Occupational Safety and Health Administration (OSHA) scrutiny and criticism without a limit. Interestingly, it was noted in this submission, through reference to the Consumer Product Safety Commission, that second or third-degree burns have been shown to occur in the elderly at temperatures not much over 43°C (110°F). Council I and the General assembly of voting delegates passed a recommendation to lower the regulatory water temperature minimum to 29.5°C (85°F). In recognition of concern expressed by a number of stakeholders with regards to the issue of handwashing water temperature, the initial results of the work described in this report and the will of state voting delegates, the 2001 Food Code lowered the required handwash water temperature to 37.8°C (100°F) (US Public Health Service 2001).

The universe of food handling situations requiring effective personal hygiene spans from temporary handwash stations set up in produce fields and county fairs to advanced state of the art clean room style kitchens used to produce extended shelf life ready-to-eat foods sold at retail. In quick service restaurants, workers frequently switch between food and money handling. Due to the potential for money to carry potential pathogens, as described by Michaels, hands may require washing from up to 40 times or more in an 8-h shift (Michaels 2002). In many of these situations, it is difficult to provide water meeting strict temperature ranges. With regard to international settings, it is doubtful that underdeveloped parts of the world will easily be able to tap into warm/hot water supplies, much less into clean water sources at all. Water temperature shortcomings have been a common point of criticism by food safety experts when reviewing handwashing procedures in the developing world as part of HACCP activities. Further, no matter where the location, it is difficult to manage and monitor food handlers to insure that minimum temperature levels are maintained during all handwashing activities. When subject to regulatory inspections, in the U.S., violations are given to food industry entities based on Food Code specifications. In some cases, based on accumulation of violations with water temperature being one of them, mandatory 48 h closure can result. This appears to be both costly and unnecessary based on the results of the studies described here.

In an extensive literature review of the effect of water temperature on hygienic efficiency, only two existing experimental studies shed light on this issue. Both of these involved hand sampling studies, in which the objective was to remove, identify and enumerate as many bacteria on the hands as possible, either as normal or transient flora. In hand scrubbing experiments, Price found that at temperatures from 24°C (75.2°F) to 56°C (132.8°F) there was no difference in de-germing rate (Price 1938). Since he scrubbed hands with a brush for a specific period of time, each in turn in a series of sterile wash basins, he might have been capable of seeing differences upon counting the flora in each basin. After conducting over 80 experiments in a 9-year period, Price concluded that the largest variable in determining the rate of removal of bacteria from the hands was the vigorousness of scrubbing. Other factors such as soap used or water temperature were less important. In later hand sampling experiments by Larson and others (implementing the glove juice method for recovery of microorganisms), no differences in isolation rates were seen at either 6°C (42.8°F) or 23°C (73.4°F) (Larson *et al.* 1980). While this information is inconclusive and does not answer questions concerning bacterial loads suspended in a confounding soil, they tend to indicate that there may not be a noticeable difference in efficacy over a range of temperatures from 6°C (42.8°F) to 56°C (132.8°F).

Various menstrua have been used for handwashing efficacy studies. For studies involving transient flora, the most often used soil is tryptic soy broth (TSB). Microorganisms exhibit good survivability, with even distribution of contaminating microorganisms into skin cracks, creases and invaginations being possible. Ground beef probably represents the most appropriate menstrua because of concern for risks of *E. coli* O157:H7 infection, but is only occasionally used (Sheena & Stiles 1982; Stiles & Sheena 1985). Meade and others have shown numerous sporadic cases of foodborne illness have been tied to poor personal hygiene after ground beef preparation (Mead *et al.* 1997). In addition, due to it's viscosity, thixotrophic properties and level of organic soil, it would appear to be a good surrogate for fecal material.

A review of pertinent literature was also undertaken to determine if, independent of efficacy, facts on skin damage support a lowering of the temperature. The Consumer Product Safety Commission (CPSC) has noted that residential water heater thermostat settings should be set at 49°C (120°F) to reduce the risk of the majority of tap water scald injuries. Although the majority of scalding attributed to the home occur in children under the age of five and the elderly, third-degree burns are known to result in a two second exposure to 66°C (150°F), six-seconds at 60°C (140°F) and 30 s at 54.4°C (130°F) (US Consumer Product Safety Commission 2000). As we age, our skin becomes thinner, loosing suppleness. This fact is important as many seniors are now actively involved in the food service industry. Due particularly to the elder risk, some have recommended that water be delivered from the tap at even lower temperatures of less than 43°C (110°F) (Stone *et al.* 2000).

The activity of soaps, friction and rinsing become crucial since the temperatures recommended in handwashing water alone would not provide thermal destruction of pathogenic microorganisms. Relevant to the discomfort issue associated with hot water is a previously conducted study by Horn and Briedigkeit involving dishwashing soaps (Horn & Briedigkeit 1967). In that study, participants were only able to withstand water temperatures at 43°C, 45°C, and 49°C (110°F, 113°F and 120°F), with tolerance levels due to discomfort peaking at one-minute (Horn & Briedigkeit 1967). Even though considerably longer than the 10–25 second exposure period that would result from handwashing, it is indicative of the fact that temperatures from 43°C and upwards (110°F and upwards) are at or near the human discomfort threshold.

Friction has been described as a key element in removing microbial contaminants from hands (Price 1938; Kaul & Jewett 1981). Friction applied during hand drying is instrumental in finishing the process (Madeline & Tournade 1980; Knights *et al.* 1993; Michaels *et al.* 2002). Removal of transient flora appears to be even more friction dependent than removing resident flora. Surfactant and antimicrobial compounds in soap are responsible for lifting soil and killing microorganisms suspended in the soil. When using bland soap to wash hands, handwashing efficacy appears to be dependent on the effects of surfactant action of the soap along with friction applied during the washing and rinsing process. Rinsing also provides the necessary removal by dilution. To facilitate appropriate rinsing of the hands, some personal hygiene consultants have suggested the practice of using thicker, higher viscosity soaps in larger doses, which would require a longer, more vigorous rinsing routine.

Price, upon noticing that in his scrubbing experiments that water temperature had little effect at degerming of the skin, commented that water applied to the skin at a given temperature quickly reaches equilibrium with normal skin surface temperature unless hands are totally immersed (Price 1938).

Skin oils derived from sebum are liquid in the sebaceous gland and solidify on the skin surface. Beef tallow has a melting point range between 35°C and 40°C (95°F and 104°F), while lard or butterfat are liquefied at around 30°C (86°F) (Lide 1990). If handwashing efficacy for both resident and transient floras embedded in both natural and artificially applied fats depended on thermal melting, then log10 reduction figures should have been greatest at the highest temperature and least at temperatures causing fats and sebum to congeal.

Fats such as tallow or lard are distinguished from oils in that the latter are liquids at room temperature. Hand soap formulations are designed to lift soil through their foaming action, dispersing and solubilizing organic soils through action of detergent surfactants. Primary micelles are formed, having hydrophilic and hydrophobic groups attached to each end of the surfactant monomer. Soaps with multiple surfactants form mixed micelles, which increases efficiency with various soil mixtures. In water and organic soil mixtures, these form complex micelle structures around hydrocarbon moieties (encapsulation) resulting in microemulsions. Thus, the soap provides a 'bridge' between the oily droplet and water, permitting the soapy water to 'wash away' greasy material.

Materials and methods

The quantity of soap used for handwashing has the ability to effect handwashing efficacy, as shown by Larson (Larson *et al.* 1987). Various investigators (Michaud *et al.* 1972, 1976; Ojajarvi 1980; Stiles & Sheena 1987; Mahl 1989; Larson *et al.* 1990; Rotter & Koller 1992; Miller & James-Davis 1994; Paulson 1994) have used soap amounts in the range of 2.5–5.0 mL in their handwashing efficacy protocols. The higher levels are considered excessive, except in the area of hospital infection control. Many food service operations set soap dispensers at 1 mL per pump, and employees often times use multiple pumps. For this study, 3 mL of soap was chosen to represent an amount found to be significantly effective in an earlier study described (Larson *et al.* 1987).

Determination of appropriate handwashing duration for these studies (15 s) was arrived at through review of various governmental regulatory standards, test method guidelines and food safety specialist recommendations along with previous handwashing study observations. Suggested lathering times by specific entities are: The 1999 FDA Food Code (US Public Health Service 1999) (20 s), The American Society for Testing and Materials (American Society for Testing and Material 1995) (15 s), The Association for Professionals in Infection Control and Epidemiology (APIC) (Jennings & Manian 1999) (minimum of 10 s), and The American Society for Microbiology (American Society For Microbiology 1996) (a 10–15 second vigorous scrub). Several studies support a washing duration of at least 10 s, with sufficient transient removal efficiency achieved by 30 s. A study by Stiles and Sheena involving workers in a meat processing facility determined that a wash of 8–10 s was too short for adequate soil removal from the hands (Stiles & Sheena 1987). A study by Ojajarvi compared a 15 second and 2 minute wash, with the latter providing only an additional 3% transient bacterial reduction (Ojajarvi 1980). One observational study in food service indicates average duration times of 20 s in a silver service restaurant kitchen (Ayers 1998).

In our first study, the effects of water temperature on the reduction of both resident (normal) and transient bacteria during handwashing was performed at each of the following temperatures: 4.4°C (40°F), 12.8°C (55°F), 21.1°C (70°F), 35°C (95°F), or 48.9°C (120°F). Two separate laboratories participated in this work. Silliker Laboratories (South Holland, IL, USA) was responsible for transient flora experiments while BioScience Laboratories (Bozeman, MI, USA) performed normal flora studies. For transient flora studies, the experimental subjects' hands were artificially contaminated with Serratia marcescens in Tryptic Soy Broth (TSB) or irradiated ground hamburger. Sterile, uninnoculated TSB and irradiated ground hamburger were used as confounding soils in testing for the reduction of the resident flora. Following hand contamination, baseline microbial counts were acquired using the 'glove-juice' method on one hand. Hands were moistened and washed/lathered for 15 seconds with 3 mL bland (nonantibacterial) soap, rinsed for 10 seconds (water flow rate of 7 L/minute) at the assigned water temperature (also used for the prelather moistening), and the opposing hand was then sampled using the same glove-juice technique. No drying of hands was performed, which would have had the effect of diminishing differences between experimental groups. Baseline and postwash readings were then compared to obtain bacterial reduction values. For this study, no skin condition assessments were performed.

The first study was performed using a non-antibacterial soap and examined temperature effects on bacterial reductions based on the solubility of greasy soils. It did not address the increased temperature effect on antimicrobial activation or possible skin damage. Therefore, the second study was undertaken, which not only involved a comparison of the microbial reduction effects of four antibacterial soaps at two different temperatures, but also evaluated skin conditions on the hands of participants throughout the study. The potential of each soap to cause negative skin changes at each water temperature combination was assessed by measuring the skin moisture content, rate of water loss from the skin, skin scaliness by computerized analysis of a digitized skin image, and by visual assessment of the dryness and erythema. This study was performed at BioScience Laboratories, employing eight subjects and using four different antimicrobial soaps, each having a different antimicrobial active ingredient. The soaps had antimicrobial activity equivalent to USDA E2 ratings (50-p.p.m. chlorine equivalency). The active ingredients in these products were Quaternary Ammonium (3% dual Quat formulation), Triclosan (1%), Parachlorometaxylenol (PCMX-3%), and Iodophor (7.5% PVP-I). Participants consisting of paid volunteers performed multiple handwashes during two five-day test periods (weeks one and two) seven days apart using *Escherichia coli* (ATCC #11229) contaminated gamma irradiated ground beef. On days one through five of weeks one and two, the skin condition was evaluated visually, for moisture content using the Corneometer® CM825, for total evaporative water loss using the TC350 Tewameter, and digitally using the Skin Visiometer® SV 500 with Visioscan® VC98. The visual skin dryness and erythema (redness) scoring was performed by a single blinded (unaware of subjects antimicrobial soap product/water temperature configuration) evaluator trained in assessment of skin damage or irritation using a 0–6 scoring system (see Table 2) as originally described by Griffith and others (Griffith *et al.* 1969). Log_{10} reduction data was determined with the first wash of days one, three and five under each water temperature condition. After handling the contaminated ground beef in a way to uniformly contaminate hands, one hand was sampled immediately (again, using the 'glove-juice' technique) for a baseline reading. The subjects' then washed both hands at the specific water temperature (85° ± 2°F for week one and 110° ± 2°F for week two) with their randomly assigned product with their opposing hand being sampled to establish microbial counts. Each subject then washed 11 consecutive times with their assigned test product each day drying hands between washes, then hands were evaluated visually and digitally 30 minutesfol-

Table 2 Grading scale for evaluating the skin of the hands*

Grade	Description
0	No visible damage, 'perfect' skin
1	Slight dryness, ashen appearance, usually involving dorsum only
2	Marked dryness, slight flaking involving dorsum only
3	Severe dryness dorsum, marked flaking, possibly fissures in webs
4	Severe flaking dorsum, surface fissures possibly with slight palmar dryness
5	Open fissures, slight erythema (>10% of dorsal and interdigital surface), with or without severe dryness, no bleeding
6	Bleeding cracks, deep open fissures, or generalized erythema (>25% of area)

*Griffith *et al.* 1969.

lowing the last wash. In all washing cases, lathering was performed for 15 seconds and rinsing for 10 seconds with three mL of the assigned test product.

Results and discussion

After extensive statistical analysis of the results from the first set of experiments, it was determined that there was no significant difference in bacterial log_{10} reductions for either resident or transient bacteria at any of the test washing and rinsing temperatures. See Figs 1 and 2 for transient and resident flora data, respectively. Average log_{10} reduction results for each soap are presented in Fig. 3.

After extensive statistical analysis of the second experiment with antibacterial soaps involving the 2 sample *T*-test, Kruskal–Wallis test and Mann–Whitney test, no statistical difference in log_{10} reductions was detected between the two wash temperatures for any of the products or as a group. Overall, the four products produced similar handwashing efficacy results. Although most of the washes at the higher temperature did produce a slight increase in bacterial reductions, it was not enough to be considered statistically significant. Figure 4 shows Tewameter® readings measuring *trans* epidermal water loss, while Figs 5 and 6 show visual dryness and baseline adjusted Corneometer® values, respectively. Skin scaliness values using a Visiometer® are shown in Fig. 7. Along with the slight additional reduction of bacteria at the higher temperature was increased skin visual dryness, increased transepidermal water loss and decreased scaliness, also determined to be statistically insignificant. Skin scaliness is highest on day one and two at the higher temperature but for days three, four and five, this reverses.

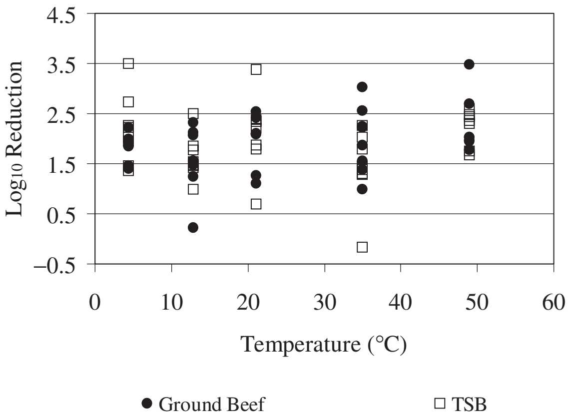

Figure 1 Handwashing efficacy (Log_{10} reduction) for transient flora (*S. marcescens*) in ground beef and TSB at selected water washing and rinsing temperatures.

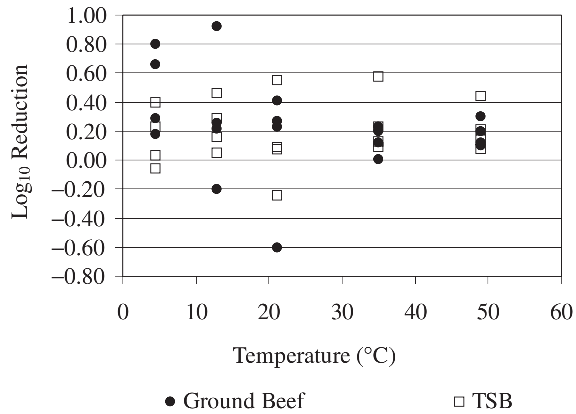

Figure 2 Handwashing efficacy (Log_{10} reduction) for resident flora in ground beef and TSB at selected water washing and rinsing temperatures.

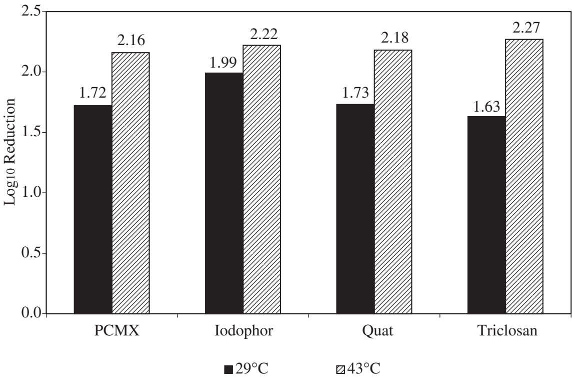

Figure 3 Average Log_{10} reduction of transient flora (*E. coli*) in ground beef using selected antimicrobial soaps.

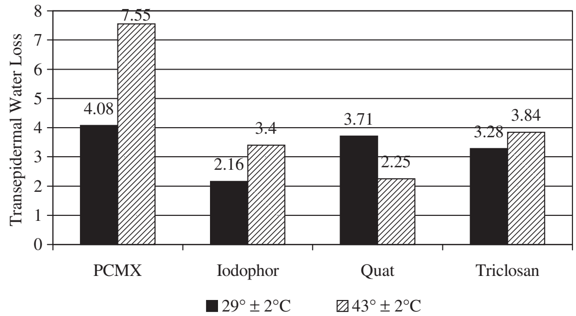

Figure 4 Average Tewameter® readings selected antimicrobial soaps at 2 different water temperatures.

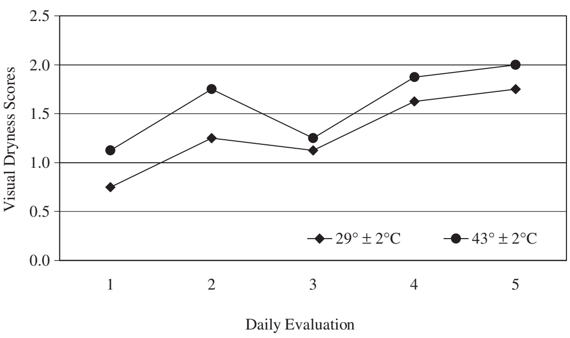

Figure 5 Average baseline-adjusted visual dryness scores (8 subjects) resulting from washing hands with 4 different E2 antimicrobial soaps for 5 days (12 ×/day).

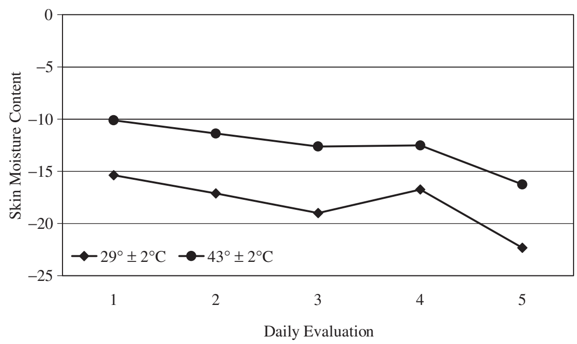

Figure 6 Baseline-adjusted Corneometer® readings (8 subjects) resulting from washing hands with 4 different antimicrobial soaps for 5 days (12 ×/day) at two different handwashing temperatures.

It is conceivable that the higher temperatures more rapidly removed loose layers of stratum corneum.

The results from both of these experiments are in agreement regarding the lack of hygienic benefits of washing hands at higher water temperatures and particularly at temperatures at the upper end of human tolerance, sometimes described as 'hot as you can stand'. From the first study, it is realized that higher water temperatures have no significant effect on the

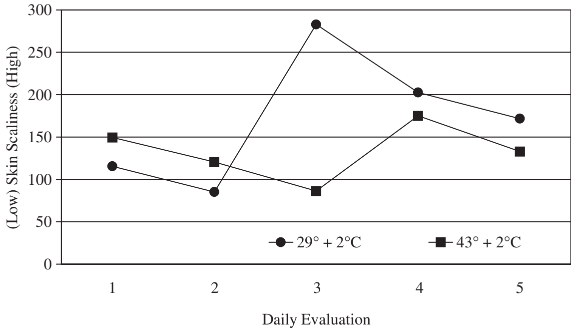

Figure 7 Average baseline-adjusted skin scaliness (8 subjects) resulting from washing hands with 4 different antimicrobial temperatures as measured using Visiometer®.

reduction of resident or transient bacteria in either easy to remove soil (TSB) or difficult to remove soil (ground beef) when using plain soap at a wide range of temperatures and using a standard hand wash. The second study provides additional support to the results of the first study by showing no statistically significant effect for the use of 110°F water (compared to 85°F water) to remove transient microorganisms embedded in ground beef from the hands when using any one of four different antibacterial based soaps or antibacterial soaps as a group. This experiment did show the trend toward higher kill as well as higher level of skin damage supporting propositions put forward by both camps. Log_{10} reductions do reflect slightly greater efficacy at higher temperatures but not at the level of significance expected, most probably due to the rapid equilibration to hand temperature described by Price (Price 1938).

Water has been identified as a skin irritant in its own rite, and part of this irritant potential can be exacerbated by temperature increase (Tsai & Maibach 1999). Repeated water exposure causes extraction or dilution of natural moisturizing factors in the stratum corneum. The water-holding property of the stratum corneum is provided in part by intercellular lipids and lipid rich sebaceous gland secretions (Noble & Pitcher 1978). The intercellular lipids, which when chromatographically fractionated, can be separated into cholesterol, cholesterol esters, phospholipids, free fatty acids, glycolipids and ceramide (Noble 1975; Imokawa *et al.* 1986). Loss of these lipid components results in a chapped and scaly skin appearance (Imokawa & Hattori 1985). Water induced irritation is known to exist in workers involved in continuous wet work, resulting in chapped and dry skin after wet work is completed (Halkier-Sorensen & Thestrup-Pedersen 1991).

Instances of primary irritant dermatitis to certain chemicals has been found to occur when hot water at 43°C (110°F) was used rather than lukewarm at 23°C–25°C (73°F–77°F) (Rothenborg *et al.* 1977). Detergent/surfactant formulations are known to cause changes to the stratum corneum such as disaggregation, swelling and morphological deterioration of corneocytes (Shukuwa *et al.* 1997). It has been found that heat plays a part in accelerating irritation of certain chemicals found in these detergent formulations. Berardesca and others found a significant difference between the temperatures of 20°C and 40°C (68°F and 104°F) in skin irritation to 5% sodium lauryl sulphate solution for a 4-day exposure period (Berardesca *et al.* 1995; Ohlenschlaeger *et al.* 1996). This irritation is documented using transepidermal water loss (TEWL) measurements, erythema (skin redness), skin reflectance, hydration (capacitance) and desquamation (stripping). Gross hand edema has been found to occur at temperatures between 35°C (95°F) and 45°C (113°F) when hands are completely immersed at those temperatures (King 1993). A significant increase in blood flow has also been shown in comparisons between 37°C and 43°C degrees (99°F and 110°F) (Nagasaka *et al.* 1987). Overall, these studies tend to show that food service workers derive no significant measurable benefit by using hot water (105°F +) to wash and rinse hands. Use of water at higher temperatures does seem to result in physiological changes collectively described as skin damage. There may be severe consequences of frequent use of hot water for handwashing at temperatures above 43°C (110°F), which can damage skin and heighten susceptibility to both allergens present in the food service environment and/or colonization (Larson *et al.* 1998). Rather, water temperature should be set at what is considered comfortable and generally conducive to handwashing.

The central components of effective handwashing thus consist of soap use in a way that promotes emulsification of soil (through vigorous friction/mechanical action) followed by thorough rinsing and drying, which again adds friction to the equation. Guidelines for handwashing in food service should probably not specify water temperature descriptors other than perhaps the word 'comfortable' when it comes to defining effective handwash standards. 'Warm' or 'tempered' would probably be acceptable, but more importantly as indicated by Jennings and Manian (1999), 'running water' should be to rinse away emulsified soils and associated transient contamination. Fingertips should be pointed down and hands rinsed and dried in a way to focus on parts of the hand that have shown to be missed during normal handwashing. This includes fingertips, thumbs and fingernail regions.

Conclusions

A review of the literature on the subject of handwashing water temperature requirements showed considerable variation with respect to expert opinion on optimal temperature for removal of microbial contaminants form hands. There in fact was a virtual absence of data to back up the various positions on the subject. Sanitarians and food safety experts have specified water temperatures varying from room temperature (running water) up to 'as hot as you can stand', the latter of which is probably in the range of from 49°C (120°F) to 55°C (131°F). Regulations in the US and elsewhere tend to focus on temperatures between 43°C (110°F) and 49°C (120°F). Concern that these temperatures could be detrimental to skin health without documented efficacy led to the experiments described here. Hands were contaminated with soils similar to those encountered in the food service environment. These soils contained marker bacteria allowing handwashing efficacy to be determined at specified water temperatures against both transient flora and resident flora simultaneously.

The initial experiment involved testing with bland non-antimicrobial soap at 5 temperatures from 4.4°C (40°F) to 49°C (120°F). Independent of soil or bacterial type (resident or transient) there was no significant difference in efficacy attributed to water temperature. In the second experiment antimicrobial soaps (4) were used having different antimicrobial active ingredients, at each of two water temperatures, 29.5°C (85°F) and 43°C (110°F). Skin condition was monitored with frequent handwashes (12 ×/day) for the second set of water washing temperature experiments. In this experiment, even though slightly higher efficacy with was seen with antimicrobial soaps at higher temperatures, overall, there was no statistical difference in efficacy as measured in Log_{10} reduction at the two water temperatures (regardless of soil or microflora types). Concomitant to the increase in efficacy at higher temperatures was a consistent trend for increases in measures of skin damage, such as skin moisture content, transepidermal water loss and erythema. This was also found not to be statistically significant.

Both the trend for higher efficacy of soaps with attendant skin damage at higher temperatures are grounded in theory. Under the conditions of these experiments neither was shown to be proven for practical application. Since efficacy is not markedly improved at higher temperatures but rather the real danger exists of skin damage, requirements for specific handwashing water temperature should be relaxed to improve acceptance of frequent handwashing by food workers at appropriate times to reduce foodborne illness potential. Water temperature should be in a comfortable range, perhaps tempered.

As has been shown by many previous researchers, overall handwashing effectiveness is more dependent on the vigorousness of execution than details such as the type of soap, the length of handwash or in this case water temperature. The results obtained in these experiments confirm the observations made by Price (Price 1938) and Larson (Larson *et al.* 1980) indicating water temperature had little or no effect on the removal of bacteria from hands. While their original reports dealt with optimizing skin sampling efficacy, for the types of experiments performed and described in the current report.

Unfortunately, food service regulatory authorities, health inspectors and environmental health officers in the US and elsewhere have fixated on handwashing water temperature because it is measurable and in the somewhat mistaken belief that higher temperatures would result in cleaner hands. Up until recently, the existence of adequate hygiene facilities (functioning toilet, toilet paper, functioning sink, soap and paper towels) and water temperature measurement were to some extent the only measurable qualities whereby food safety inspectors could cite food service facilities for violation. Poor personal hygiene is often used after the fact to describe as a contributing factor aiding to an outbreak. With handwash monitoring devices employees' handwashing can be monitored, documented and verified within the HACCP framework (Michaels 2002). With this new technology and information from this report indicating that water temperature for handwashing is relatively unimportant, perhaps regulatory authorities will be able to focus on other more important factors having a bigger impact on food safety.

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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

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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] * $P < 0.05$.

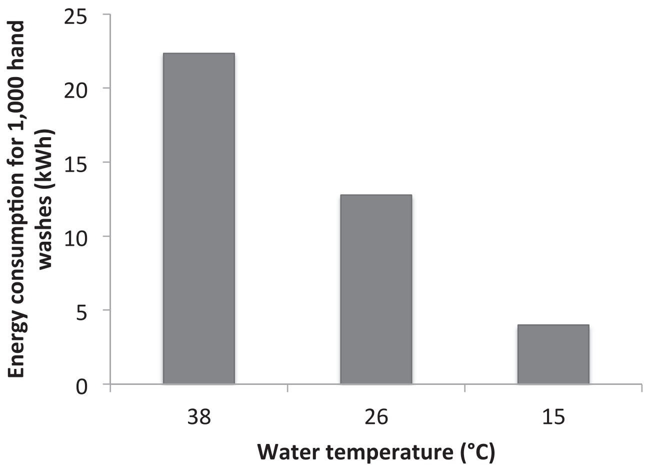

FIGURE 1. *Energy consumption related to water heating for hand washing.*

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): Lather	Rinse	Total	% volunteers using: 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%; $P < 0.05$) 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.

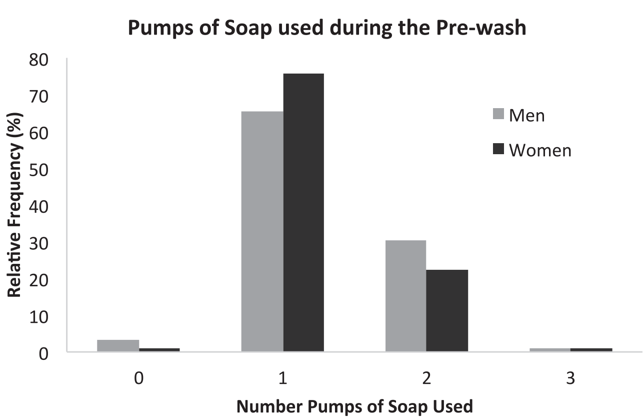

FIGURE 2. *Number of pumps of soap used by women (solid) and men (shaded) during the prewash. Each pump delivered 0.5 mL of soap.*

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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