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Alternative Hand Contamination Technique To Compare the Activities of Antimicrobial and Nonantimicrobial Soaps under Different Test Conditions[▽]

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Antimicrobial hand soaps provide a greater bacterial reduction than nonantimicrobial soaps. However, the link between greater bacterial reduction and a reduction of disease has not been definitively demonstrated. Confounding factors, such as compliance, soap volume, and wash time, may all influence the outcomes of studies. The aim of this work was to examine the effects of wash time and soap volume on the relative activities and the subsequent transfer of bacteria to inanimate objects for antimicrobial and nonantimicrobial soaps. Increasing the wash time from 15 to 30 seconds increased reduction of *Shigella flexneri* from 2.90 to 3.33 $\log_{10}$ counts ($P = 0.086$) for the antimicrobial soap, while nonantimicrobial soap achieved reductions of 1.72 and 1.67 $\log_{10}$ counts ($P > 0.6$). Increasing soap volume increased bacterial reductions for both the antimicrobial and the nonantimicrobial soaps. When the soap volume was normalized based on weight (~3 g), nonantimicrobial soap reduced *Serratia marcescens* by 1.08 $\log_{10}$ counts, compared to the 3.83-$\log_{10}$ reduction caused by the antimicrobial soap ($P < 0.001$). The transfer of *Escherichia coli* to plastic balls following a 15-second hand wash with antimicrobial soap resulted in a bacterial recovery of 2.49 $\log_{10}$ counts, compared to the 4.22-$\log_{10}$ ($P < 0.001$) bacterial recovery on balls handled by hands washed with nonantimicrobial soap. This indicates that nonantimicrobial soap was less active and that the effectiveness of antimicrobial soaps can be improved with longer wash time and greater soap volume. The transfer of bacteria to objects was significantly reduced due to greater reduction in bacteria following the use of antimicrobial soap.

Hand washing has long been considered one of the easiest and simplest public health practices for preventing the spread of disease in clinical and nonclinical settings (2, 10, 12, 14, 25). Recommendations on wash times and the proper procedure for washing hands have been published by various public health organizations (6, 37). The transmission of transient bacteria by the hands plays a significant role in direct and indirect transmissions of disease. While experts agree that hand washing with soap and water is effective at reducing the spread of disease-causing bacteria, there still remain doubts on the benefit of antimicrobial hand washes over nonantimicrobial soap and water. Studies looking at the reduction of disease between groups using antimicrobial soap and those using nonantimicrobial soap continue to show conflicting results (1, 18, 30). The differences in findings may be due to confounding factors, such as soap volume, wash time, type of antimicrobial product, and lack of uniformity among these factors in the published studies.

The effects of these variables on assessment of hand washing efficacy have also been studied. Larson et al. demonstrated that soap volume and wash time can have an effect on the numbers of resident bacteria remaining on the hands after multiple hand washes but not after a single hand wash (17). The effect on transient gram-negative bacteria was not investigated. Other studies have looked at the role that hand contamination techniques can have in evaluating relative antimicrobial activity. Using *Staphylococcus aureus* as a transient marker organism, Lilly and Lowbury looked at the effect of drying the bacteria on the fingertips compared to that of rubbing the bacteria into the fingers on activity (20). A number of variables, including differences in delivery systems (liquid and bar soaps), amounts of product used, and products to be used with and without water (alcohol), make evaluating the results difficult. Another study (28) compared the effect of rubbing the inoculum into the hands versus that of simple drying on activity and concluded that the hand contamination method was not a determining factor in evaluating efficacy. None of these studies used consistent methodologies or looked at the subsequent transfer of bacteria remaining on the hands after washing as a way of evaluating and comparing the activities of hand washes.

Antimicrobial and nonantimicrobial hand wash activities have also been compared, and antimicrobial hand wash products have been shown to provide greater bacterial reductions than nonantimicrobial soaps (26, 32). However, direct comparisons between these studies and others are not possible, due to differences in methodology. Whether this additional reduction results in fewer illnesses has not been definitively demonstrated. In order to evaluate the antimicrobial activities of hand wash agents under controlled conditions, a standard method, the American Society for Testing and Materials (ASTM) E1174 method (Standard Method for Evaluation of Health Care Personnel Hand Wash Formulations), is used (3). Comparisons between nonantimicrobial soaps and antimicrobial soaps continue to be measured based on bacterial reduction alone and not subsequent transfer of bacteria following

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use. Current methods do not provide a means to evaluate the transmission of bacteria to objects following hand washing. While many aspects of the ASTM test have been standardized, such as hand contamination and bacterial recovery procedures, they are not based on clinical models or use patterns. Other variables, such as baseline inoculum concentration, wash and rinse treatment time, and volume of test material, have not been standardized (3). Studies have demonstrated the potential for the transfer of microorganisms from the hands to food, objects, and surfaces and also from contaminated objects to the hands (4, 7, 21, 24, 31, 38). Again, these studies did not compare the differences in transfer of bacteria to food or other objects following hand washing between nonantimicrobial and antimicrobial soap in a single study. Developing new techniques to better understand and evaluate the relative effectiveness of hand wash products will provide meaningful and useful data to help reduce the incidence of disease by hand transmission.

The goals of this study were to utilize the ASTM E1174 method but develop a more appropriate and realistic hand contamination technique, to compare how wash time and soap volume affect the relative activities of antimicrobial and nonantimicrobial soaps under various test parameters, and to measure the subsequent transfer of transient bacteria to objects following hand washing with these products.

MATERIALS AND METHODS

Subjects. An institutional review board approved all protocols involving human subjects, and the research complied with all federal and institutional guidelines. Healthy adult subjects were recruited to participate in each test study. The subjects were between 18 and 60 years of age. For each test, subjects were randomly assigned to a treatment group. Each subject received only one of the test treatments during the studies.

Microorganisms and growth conditions. The bacterial strains used in these studies were *Serratia marcescens* ATCC 14756, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 11229, and *Shigella flexneri* ATCC 700930. Microorganisms were obtained from American Type Culture Collection (ATCC) and were propagated according to ATCC recommendations. Stock cultures were maintained using the Microbank bacterial preservation system (Pro-Lab Diagnostics, Austin, TX) and stored at −80°C. Bacteria were grown in tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD) at 35°C for 24 h. A 24-hour broth culture was streaked onto Trypticase soy agar (TSA) (Becton Dickinson, Sparks, MD) and incubated for 24 h at 35°C. A study challenge pool was made by transferring at least three isolated colonies from the TSA plate to a sterile vessel of TSB. A series of at least three but no more than five 24-hour broth transfers was made in 10 ml of TSB. For studies where the inoculum level was $\geq 10^8$ CFU/ml, a volume of TSB that would be required for the study was inoculated with the culture and incubated at 35°C for 16 to 20 h. This inoculum was used without dilution in the studies and was typically at a titer of 1.0×10^8 to 1.0×10^9 CFU/ml. For studies where a lower inoculum level was to be used, an appropriate amount of TSB culture incubated as described above was diluted on the test day into a suitable volume of 0.85% physiological saline to obtain a titer of approximately 1.0×10^6 to 1.0×10^7 CFU/ml. Thirty milliliters of the challenge pool was dispensed into sterile 50-ml centrifuge tubes. The challenge pool was not used for more than 8 hours. The pool was assayed using standard plate count procedures for the number of organisms at the beginning and end of the use period.

Conditioning wash. A conditioning wash was performed prior to the start of each test to familiarize the subjects with the washing procedure and to remove any dirt and oil present on the hands. This helps to minimize potential soil load variability of the subjects' hands. Subjects were asked to pass their hands under running tap water tempered to 40 ± 2°C. Two pumps of nonantimicrobial soap (Johnson & Johnson Head to Toe; Skillman, NJ) were dispensed into the cupped palm of one hand. The soap was spread over the entire surface of each hand and the lower third of each forearm. Subjects washed for 15 ± 2 seconds in a vigorous fashion. The hands were rinsed under running tap water tempered to 40 ± 2°C for 30 seconds. The subjects then dried their hands thoroughly using disposable paper towels. After drying, the hands and wrists were soaked with 70% isopropyl alcohol for 30 seconds. The hands were then air dried completely. Briefly, the sequence of steps for each study was as follows. A conditioning wash was performed on all subjects. Next, the subjects' hands were contaminated using the palmar-surface technique. The hands were immediately sampled using the prescribed bacterial recovery method. The calculated mean bacterial recovery represents the baseline value for each treatment group. Following the baseline sampling, the subjects' hands were washed and soaked with isopropyl alcohol as described for the conditioning wash. After air drying, the subjects' hands were again contaminated using the palmar method. The hands were then washed using the assigned treatment product. After wash treatment, either one or both of the hands were sampled for bacterial recovery. During the hand transfer studies, the dominant hand was used to handle the plastic balls while the nondominant hand was sampled for bacterial recovery.

Hand contamination: palmar-surface technique. Two single-ply paper towels (Brawny Light-Duty; Georgia Pacific) were folded together into a rectangle approximately 12.7 cm by 21.6 cm. The towels were placed inside an aluminum foil pouch and sterilized by autoclaving. Just prior to subject contamination, one pouch for each hand was opened, exposing the paper towel. A 30-ml bacterial suspension was poured evenly onto the towel, allowing for the complete absorption of the suspension. The subjects' hands were placed directly above the individual towels and then pressed down firmly for 5 ± 1 seconds, ensuring that the entire palms, fingers, and finger pads were in contact with the saturated towel. The hands were then air dried for 90 ± 5 seconds, followed by a standard bacterial recovery method or hand treatment as described below.

Bacterial recovery method. Plastic bags (Glad food storage bags or equivalent; 29.2 cm by 31.8 cm) were placed on the subjects' right and/or left hands. Aliquots (75 ± 2 ml) of stripping solution with neutralizer (0.075 M phosphate buffer with 0.1% Triton X-100) were added to each bag. The bag was secured at the wrist, and the hands were massaged for 1 minute in a uniform manner. A 1-ml aliquot was obtained from the bagged hands within 1 minute of completing the massage and placed into a sterile tube for further dilution and plating.

Enumeration of bacteria from hands and plastic balls. Serial 10-fold dilutions were performed with Butterfield's phosphate-buffered water by using the initial 1-ml aliquot from the bagged hands or plastic balls. Dilution aliquots were plated using standard plate count procedures (35). Hektoen enteric agar (Difco) was used for *S. flexneri*, MacConkey agar (Difco) was used for *E. coli*, and TSA was used for *S. marcescens* and *S. aureus*. All plates except those for *S. marcescens* were incubated at 35 ± 2°C for 36 to 48 h. *S. marcescens* was incubated at 25 ± 2°C for 36 to 48 h to enhance pigmentation development. Plates yielding 25 to 250 colonies were counted using standard plate-counting procedures.

Hand treatment with test article. Prior to hand treatment, the subjects' hands were contaminated as described above. Following the hand contamination, the subjects were instructed to perform a hand washing treatment specific to each type of hand soap tested. The subjects spread the material over the entire surface of each hand, including the back of the hand, between the fingers, and the lower third of the forearm. The soap was rubbed vigorously over the hands for 15 or 30 seconds, and then the hands were rinsed under tap water tempered to 40 ± 2°C for 30 seconds. Either the hands were immediately sampled using the above-described bacterial recovery method or the subjects were asked to handle plastic balls. The hands were not dried prior to either sampling or handling plastic balls.

Test articles. The test articles used to evaluate the effects of wash time (Table 2) were foaming antimicrobial hand soap containing 0.46% triclosan (Dial Complete antibacterial foaming hand wash; Scottsdale, AZ) and nonantibacterial foaming hand soap (Kiss My Face self-foaming hand wash; Gardiner, NY). The test articles used to evaluate the effects of soap volume and transfer of bacteria (Tables 3 and 4) were foaming antimicrobial hand soap containing 0.46% triclosan (Dial Complete antibacterial foaming hand wash; Scottsdale, AZ) and nonantibacterial foaming soap (Wash Suds Honey Pot; Gardiner, NY). The volume of soap used in each study is described by the number of pumps, based on prescribed label use, and also in grams per pump.

Bacterial transfer to plastic balls. Prior to the test day, plastic balls, 20 mm in diameter (Techne, Burlington, NJ), were placed in a glass beaker and autoclaved for 15 min at 121°C. Four plastic balls per subject were placed into a sterile specimen cup (100-ml capacity; VWR). Following hand treatment with the prescribed test material, the plastic balls were dispensed into the subjects' dominant cupped hand. The subjects rolled the balls in their palms by using the thumb and fingers for 15 ± 2 seconds. The plastic balls were placed into sterile bags, and 20 ml of stripping solution with neutralizer was added to the bag. The bags and stripping solution were agitated by hand for 30 seconds. Bacterial enumeration was performed using standard plating methods (35).

TABLE 1. Recovery of bacteria from hands after palmar surface contamination[a]

Bacterial strain	Avg $\log_{10}$ recovery/hand (95% CI) (no. of hands)	
	Low inoculum ($\sim 10^6$ CFU/ml)	High inoculum ($\sim 10^8$ CFU/ml)
Serratia marcescens	6.42 (6.34–6.53) (48)	8.02 (7.94–8.10) (17)
Staphylococcus aureus	6.01 (5.91–6.11) (30)	NA
Escherichia coli	5.81 (5.70–5.92) (30)	NA
Shigella flexneri	6.03 (5.96–6.10) (32)	NA

[a] All cultures were obtained from ATCC. NA, not applicable.

Calculations and statistical analysis. The number of bacteria per hand was calculated by multiplying the number of CFU/ml obtained in the plate count by 75, the volume in milliliters of stripping solution used in the bag. The number of CFU/hand was then converted to $\log_{10}$ counts. For Table 1, the mean $\log_{10}$ recovery/hand was calculated by averaging the values for each hand sampled. Baseline values (Tables 2, 3, and 4) were calculated by first averaging the right and left hand bacterial $\log_{10}$ counts for each subject and then averaging the $\log_{10}$ counts for all subjects within a treatment group (antimicrobial or nonantimicrobial hand wash). The baseline values from each group were compared using an analysis of variance (ANOVA) with an α of 0.05. The ANOVA showed that the baseline values of the treatment groups were not statistically different from one another at a 95% confidence level. The mean $\log_{10}$ bacterial reduction from a single wash was calculated by subtracting the average recovery value from the average baseline within that treatment group. For Table 4, the hand that did not handle the plastic balls was used to determine the $\log_{10}$ reduction/hand from a single wash. The number of bacteria transferred to the balls was calculated by multiplying the number of CFU/ml obtained in the plate count for the plastic balls by 20, the volume in milliliters of stripping solution used in the bag. This value was converted to $\log_{10}$ counts and reported as the $\log_{10}$ count/four balls. Comparisons of reduction and recovery values were done with ANOVAs. The *P* values from these tests are shown in the tables and in the text. A *P* value less than 0.05 indicates that the values being compared are statistically significantly different at a 95% confidence level. The 95% confidence interval for each value was also calculated and is shown next to each value in the tables.

RESULTS

Technique of palmar-surface hand contamination. A technique for contaminating only the palmar surfaces of the hands was used as described in Materials and Methods. The palmar-surface contamination technique was tested on separate test days by using four bacterial strains at a low-level (10^6 CFU/ml) starting inoculum and was also tested at a high-level (10^8 CFU/ml) starting inoculum using *S. marcescens*. Baseline counts were calculated for each hand. The average $\log_{10}$ recovery per hand was found to be reproducible between hands with all strains tested, regardless of whether the starting inoculum level was low or high (Table 1) (confidence interval of 95%). Statistically significant hand-to-hand variation was not observed between subjects (data not shown). These findings support the use of this palmar hand contamination method as an alternative to the current ASTM E1174 whole-hand contamination method. These data also show that different contamination levels can be utilized by modification of the starting inoculum level.

Effects of wash time and hand soap volume on the reduction of bacteria on the hands. E1174 test variables such as wash time and soap volume are not standardized and may have an impact on the reduction of bacteria from the hands. To assess the impact of wash time on the effectiveness of an antimicrobial soap or a nonantimicrobial soap at reducing bacteria from the hands, washes were performed using either a 15- or a 30-second wash. Subjects were randomly assigned to wash with either a foaming plain hand soap (Kiss My Face self-foaming hand wash; Gardiner, NY) or a foaming antimicrobial hand soap containing 0.46% triclosan (Dial Complete antibacterial foaming hand wash; Scottsdale, AZ) for 15 or 30 seconds. Both treatment groups were washed with the same amount of soap, approximately 3 g (two pumps or four pumps). Bacterial samples were taken from each group at baseline and after washing with either the nonantimicrobial or the antimicrobial soap. The average $\log_{10}$ reduction/hand from the baseline was calculated. The antimicrobial soap demonstrated greater bacterial log reduction than the nonantimicrobial soap at each wash time (Table 2). The antimicrobial soap reduced *S. flexneri* by 2.90 $\log_{10}$ counts at 15 seconds and 3.33 $\log_{10}$ counts ($P = 0.086$) after a 30-second hand wash (Table 2). The nonantimicrobial soap showed a 1.72-$\log_{10}$ reduction at 15 seconds and a 1.67-$\log_{10}$ reduction after 30 seconds ($P > 0.6$) (Table 2). These data indicate that washing the hands longer with nonantimicrobial soap provided no additional benefit, whereas the antimicrobial soap provided a greater reduction initially over the nonantimicrobial soap and improved with increased wash time.

The amount of soap used for washing was evaluated to determine how this variable affects the reduction of bacteria on the hands. Different volumes, determined by the number of pumps, of a foaming plain hand soap (Wash Suds Honey Pot; Gardiner, NY) and a foaming antibacterial hand soap (Dial Complete antibacterial foaming hand wash; Scottsdale, AZ) were compared. Three experiments were carried out over three test days. Subjects washed their hands for 15 seconds with either a nonantimicrobial soap (one, two, or four pumps)

TABLE 2. Effect of wash time on the reduction of *Shigella flexneri* on the hands

Test	Wash time (s)	Treatment (no. of subjects)	$\log_{10}$ count/hand (95% CI)	
			Baseline[a]	Single-wash reduction
1	15	Antimicrobial (8)	6.16 (6.03–6.29)	2.90 (2.40–3.40)[b,c]
		Nonantimicrobial (8)	6.13 (6.04–6.22)	1.72 (1.56–1.88)[b,d]
2	30	Antimicrobial (10)	5.99 (5.89–6.09)	3.33 (3.04–3.62)[b,c]
		Nonantimicrobial (10)	5.97 (5.81–6.13)	1.67 (1.43–1.89)[b,d]

[a] $P > 0.5$ (ANOVA; antimicrobial baseline versus nonantimicrobial baseline within each test).
[b] $P < 0.001$ (ANOVA; single wash; antimicrobial versus nonantimicrobial soap within each test).
[c] $P = 0.086$ (ANOVA; antimicrobial soap; test 1 versus test 2).
[d] $P > 0.6$ (ANOVA; nonantimicrobial soap; test 1 versus test 2).

TABLE 3. Effect of soap volume on the reduction of *Serratia marcescens* on the hands

Test	Soap vol		Treatment (no. of subjects)	Log_{10} count/hand (95% CI)	
	No. of pumps	Wt (g)		Baseline[a]	Single-wash reduction
1	1	1.5	Antimicrobial (12)	6.32 (6.10–6.54)	3.15 (2.78–3.52)[b,c]
		0.75	Nonantimicrobial (12)	6.34 (6.20–6.48)	0.25 (0.19–0.31)[b]
2	2	3	Antimicrobial (10)	6.24 (6.01–6.47)	3.83 (3.58–4.08)[b,c,d]
		1.5	Nonantimicrobial (9)	6.25 (5.94–6.56)	0.88 (0.61–1.15)[b,e]
3	4	3	Nonantimicrobial (10)	6.13 (5.97–6.29)	1.08 (0.75–1.41)[d,e]

[a] $P > 0.5$ (ANOVA; antimicrobial baseline versus nonantimicrobial baseline within each test).
[b] $P < 0.001$ (ANOVA; antimicrobial wash versus plain-soap wash within each test).
[c] $P < 0.001$ (ANOVA; antimicrobial soap; test 1 versus test 2).
[d] $P < 0.001$ (ANOVA; antimicrobial soap test 2 versus nonantimicrobial soap test 3).
[e] $P = 0.19$ (ANOVA nonantimicrobial soap; test 2 versus test 3).

or an antimicrobial soap (one or two pumps). The antimicrobial soap reduced *S. marcescens* by 3.15 log_{10} counts with one pump and 3.83 log_{10} counts with two pumps ($P < 0.001$) (Table 3). Increasing the nonantimicrobial soap volume from one to two pumps resulted in minimal log_{10} reductions of 0.25 and 0.88 ($P < 0.001$) (Table 3). When the volume was normalized based on delivery weight amounts of 1.5 g and 3 g (two and four pumps of nonantimicrobial soap versus one and two pumps of antimicrobial soap), nonantimicrobial soap reduced *S. marcescens* by 0.88 and 1.08 log_{10} counts ($P = 0.19$), compared to a significant increase in reduction, from 3.15 to 3.83 log_{10} counts, caused by the antimicrobial soap ($P < 0.001$) (Table 3). These findings show that increased volumes of an antimicrobial soap will result in a greater increase in bacterial reduction than increased volumes of a nonantimicrobial soap. The nonantimicrobial soap used at twice its recommended label dose (four pumps) achieved only a 1.08-log_{10} reduction.

Transfer of bacteria from the hands to an inanimate object. Studies have demonstrated that disease is transmitted not only through direct hand-to-hand contact but also through shared inanimate objects (8, 29). Therefore, we assessed the transfer of *E. coli* from the hands to an object after 15-second washes with plain hand soap and antimicrobial hand soap. The baseline counts were 8.02 log_{10}/hand for both groups ($P > 0.5$) (Table 4). The subjects' hands were contaminated a second time, and the subjects washed their hands with either the nonantimicrobial or the antimicrobial soap. The subjects were then asked to handle four sterilized plastic balls, which were then sampled for bacterial counts as described in Materials and Methods. Immediately after handling the plastic balls, the subject's other hand, the nondominant hand, was sampled to determine how many bacteria remained after washing. Consistent with previous findings, there were significantly fewer bacteria left on the hands after washing with an antimicrobial soap than after washing with a nonantimicrobial soap. The average log_{10} recovery/hand with the nonantimicrobial soap was 5.35, and that with the antimicrobial soap was 3.83 ($P < 0.001$) (Table 4). The recovery of bacteria from the plastic balls was 4.22 log_{10} counts/four balls for the group who washed with nonantimicrobial soap and 2.4 log_{10} counts/four balls ($P < 0.001$) for the group who washed with antimicrobial soap (Table 4). The use of antimicrobial soap resulted in significantly fewer bacteria remaining on the hands after washing, and therefore, fewer bacteria were transferred to the object.

DISCUSSION

Hand washing is an important health measure, and improper hand washing has been linked to illness (15, 19, 36). The transfer of bacteria from the hands to food, objects, or people plays an important role in the spread of disease (5, 15, 19, 36). Well-controlled studies in the health care setting and home setting pose numerous challenges, which can affect the findings. The test parameters used in published studies have not been consistent, and therefore, the effect that they may have had makes drawing definitive conclusions on the comparative activities of hand wash products problematic. The development of a more realistic hand contamination technique than the one described in ASTM E1174 allows for the evaluation of the transfer of bacteria from hands to objects following hand washing. In the ASTM E1174 method, the entire hand is contaminated with bacteria by pipetting the bacterial culture into cupped hands and then spreading it over the front and back of both hands. This method allows differences in the bacterial reduction levels of soaps to be compared quite effectively. However, this whole-hand contamination technique does not

TABLE 4. Transfer of *Escherichia coli* from the hands to plastic balls

Test	Treatment (no. of subjects)	Log_{10} count/hand (95% CI)		
		Baseline[a]	Single-wash recovery[b]	Transfer to 4 balls[c]
1	Antimicrobial (8)	8.02 (7.94 to 8.10)	3.83 (3.23–4.43)	2.49 (1.84–3.14)
2	Nonantimicrobial (8)	8.02 (7.94 to 8.10)	5.35 (5.10–5.60)	4.22 (3.98–4.46)

[a] $P > 0.5$ (ANOVA; antimicrobial baseline versus nonantimicrobial baseline within the test).
[b] $P < 0.001$ (ANOVA; single wash; antimicrobial versus nonantimicrobial soap).
[c] $P < 0.001$ (ANOVA; transfer; antimicrobial versus nonantimicrobial soap).

model how hands are naturally contaminated, where typically it is only the palms of the hands or the fingers that contact an object or surface. Furthermore, the whole-hand contamination method makes it difficult to study the transfer of bacteria from the hands to objects since only bacteria on the palmar surfaces of the hands will be transferred, but bacteria present on the back of the hand from the contamination step will be included in the bacterial recovery step. This masks differences in the bacterial transfer that may have occurred. The palmar method allows for differences in bacterial transfer to be measured after washing with different soaps effectively. This hand contamination technique proved to be reproducible over a number of experiments with four different bacterial strains and two different inoculum levels (Table 1). Because the low-level inoculum was diluted with saline, it contained less organic material than the high-level inoculum delivered in an undiluted broth medium. The possible effect that this reduced organic load may have had on the activities of the tested products was not evaluated but was felt to be minimal due to the surfactant levels of the test products. The palmar contamination technique allowed for both the evaluation of bacterial reduction and direct comparison of the transfer of bacteria to plastic balls following hand washing with antimicrobial or nonantimicrobial soap. It has been shown that bacteria may be more effectively transferred from wet or moist surfaces than from dry ones; however, although the subjects' hands were not dried prior to either sampling or handling plastic balls under these test conditions, hand washing with an antimicrobial soap reduced the number of bacteria that were transferred to the plastic balls by almost 2 $\log_{10}$, compared to that with a nonantimicrobial soap. This difference was statistically significant. Perhaps more importantly, the difference in levels of bacteria recovered from the plastic balls could be used to estimate the relative risks of illness and infection based on known dose responses of specific bacteria. While the transfer rates of both the antibacterial and the nonantibacterial soaps were comparable, the significant differences in bacterial reduction on hands and bacterial recovery from the balls demonstrate the potential benefit of washing with an antibacterial soap. A study using the hand contamination technique presented in this paper found that hand washing with an antimicrobial soap could have the potential to reduce disease transmission from *Shigella* spp. and *E. coli* better than washing with a nonantimicrobial soap due to fewer bacteria being transferred to food following handling (11). Additional published dose responses for *S. flexneri*, *E. coli* O157, and *Campylobacter jejuni* support the findings, based on the data presented in this study, that the number of bacteria transferred when using an antimicrobial soap will result in lower infection rates in clinical and nonclinical settings (9, 13, 22, 34). Hand washing for 15 to 20 or more seconds is recommended by most health care professionals and public health organizations (6, 16). The current ASTM E1174 method specifies a wash time of 30 seconds. Studies have shown that the average observed hand washing both in the hospital setting and in public restrooms is far less than 15 seconds (23, 27, 33). The use of both 15- and 30-second hand wash times in this study ensured that the evaluations covered the recommended times and allowed for proper lathering and coverage of all hand surfaces rather than what may be considered unacceptable practices.

Comparisons in efficacy following 15- and 30-second hand washes showed that the nonantimicrobial soap reduced *E. coli* by 1.72 and 1.67 $\log_{10}$ counts ($P > 0.6$). No additional bacterial reduction was observed with the increase in wash time. Doubling the wash time for the antimicrobial hand soap resulted in an increase in bacterial reduction from 2.90 to 3.33 $\log_{10}$ counts ($P = 0.086$). This indicates that the activity and potential effectiveness of an antimicrobial hand soap can improve with a longer wash time. Comparisons of soap volume also showed a greater effect in bacterial reduction with an antimicrobial soap than with a nonantimicrobial soap. For these experiments, quantity of soap was measured in number of pumps of soap from the dispenser. The weight of soap delivered from each pump was measured in grams. The antimicrobial soap container dispensed twice as much product in a single pump (1.5 g) as the nonantimicrobial soap container (0.75 g). When the volume was normalized based on delivery weight amount by doubling the antimicrobial soap volume, from one pump (1.5 g) to two pumps (3 g), there was a significant increase in the reduction of bacteria from the hands (Table 3). However, increasing the volume of nonantimicrobial soap from two pumps (1.5 g) to four pumps (3 g) did not significantly change the observed reduction (Table 3). The bacterial reduction of the nonantimicrobial soap increased when the volume was increased from one pump (0.75 g) to two pumps (1.5 g) (Table 3); however, the data suggest that there is a maximum level of reduction and, consequently, activity that can be achieved, and there may not be any additional benefit by further increasing the volume or wash time of a nonantimicrobial soap. The level of bacterial reduction caused by nonantimicrobial soap is due to its surfactants, which physically remove bacteria. Once maximum removal is achieved, soap amount and wash time do not improve surfactancy. Antimicrobial soap provides both surfactancy and biocidal modes of action. The authors note that different antimicrobial actives and surfactants may produce results different from those observed with these test products. However, comparisons of relative activity could be made if testing were done under these same conditions with the palmar hand contamination method. These data demonstrate that under these test conditions there are statistically significant differences in reduction of bacteria between washing with antimicrobial soap and washing with nonantimicrobial soap. Washing for longer times and increasing the amount of antimicrobial soap used can further increase bacterial reduction.

Although some studies have concluded that there is no difference in bacterial reduction and consequently no difference in health benefit between antimicrobial and nonantimicrobial soaps (1), our data suggest that bacterial reductions are significantly affected by wash time, product type, and soap volume and that the benefit of this greater reduction can be further demonstrated by the fact that fewer organisms are transferred to objects or food by the washed hands. The palmar hand contamination technique described above provides a very useful tool for understanding how hand hygiene can affect the spread of disease in clinical and nonclinical settings and the transmission of food-borne or other hand-transmitted illnesses. Further work should be done on exploring the incidence of illness caused by the ingestion of food that has been handled with hands washed with nonantimicrobial or antimi-

crobial soap. Based on the results of these experiments, both the mechanics of the hand wash procedure and the material used for hand washing can affect the potential for disease transmission and acquisition.

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REFERENCES

1. **Aiello, A. E., E. L. Larson, and S. B. Levy.** 2007. Consumer antibacterial soaps: effective or just risky? Clin. Infect. Dis. **45**(Suppl. 2)**:**S137–S147.
2. **Akyol, A., H. Ulusoy, and I. Ozen.** 2006. Handwashing: a simple, economical and effective method for preventing nosocomial infections in intensive care units. J. Hosp. Infect. **62:**395–405.
3. **ASTM International.** 2002. Standard test method for evaluation of the effectiveness of health care personnel or consumer hand wash formulations. ASTM Standard E1174. ASTM International, West Conshohocken, PA.
4. **Bidawid, S., J. M. Farber, and S. A. Sattar.** 2000. Contamination of food by food handlers: experiments on hepatitis A virus transfer to food and its interruption. Appl. Environ. Microbiol. **66:**2759–2763.
5. **Boyce, J. M.** 2001. MRSA patients: proven methods to treat colonization and infection. J. Hosp. Infect. **48**(Suppl.)**:**S9–S14.
6. **Centers for Disease Control and Prevention.** 2007. Controlling the spread of infections in evacuation centers: facts for residents about diseases that cause diarrhea and/or vomiting. Centers for Disease Control and Prevention, Atlanta, GA. http://www.bt.cdc.gov/disasters/disease/pdf/infectevac.pdf.
7. **Chen, Y., K. M. Jackson, F. P. Chea, and D. W. Schaffner.** 2001. Quantification and variability of bacterial cross-contamination rates in common food service tasks. J. Food Prot. **64:**72–80.
8. **Davies, J. B. M.** 1952. Symptomless carriers in home contacts in Sonne dysentery. Br. Med. J. **2**(4777)**:**191–192.
9. **DuPont, H. L., R. B. Hornick, M. J. Snyder, J. P. Libonati, S. B. Formal, and E. J. Gangarosa.** 1972. Immunity in shigellosis. II. Protection induced by oral live vaccine or primary infection. J. Infect. Dis. **125:**12–16.
10. **Fendler, E. J., M. J. Dolan, R. A. Williams, and D. S. Paulson.** 1998. Handwashing and gloving for food protection. Part II: effectiveness. Dairy Food Environ. Sanit. **18:**824–829.
11. **Fischler, G. E., J. L. Fuls, E. W. Dail, M. H. Duran, N. D. Rodgers, and A. L. Waggoner.** 2007. The effect of hand wash agents on controlling the transmission of pathogenic bacteria from the hands to food. J. Food Prot. **70:** 2873–2877.
12. **Guzewich, J., and M. P. Ross.** 1999. Interventions to prevent or minimize risks associated with bare-hand contact with ready-to-eat foods. U.S. Food and Drug Administration, Rockville, MD. http://vm.cfsan.fda.gov/~ear/rterisk.html.
13. **Holcomb, D. L., M. A. Smith, G. O. Ware, Y. Hung, R. E. Brackett, and M. P. Doyle.** 1999. Comparison of six dose-response models for use with foodborne pathogens. Risk Anal. **19:**1091–1100.
14. **Jumaa, P. A.** 2005. Hand hygiene: simple and complex. Int. J. Infect. Dis. **9:**3–14.
15. **Kimura, A. C., K. Johnson, M. S. Palumbo, J. Hopkins, J. C. Boase, R. Reporter, M. Goldoft, K. R. Stefonek, J. A. Farrar, T. J. Van Gilder, and D. J. Vugia.** 2004. Multistate shigellosis outbreak and commercially prepared food, United States. Emerg. Infect. Dis. **10:**1147–1149.
16. **Larson, E. L.** 1995. APIC guidelines for handwashing and hand antisepsis in health care settings. Am. J. Infect. Control **23:**251–269.
17. **Larson, E. L., P. I. Eke, M. P. Wilder, and B. E. Laughon.** 1987. Quantity of soap as a variable in handwashing. Infect. Control **8:**371–375.
18. **Larson, E. L., S. X. Lin, C. Gomez-Pichardo, and P. Della-Latta.** 2004. Effect of home cleaning and handwashing products on infectious disease symptoms: a randomized, double-blind trail. Ann. Intern. Med. **140:**321–326.
19. **Lee, L. A., S. A. Ostroff, H. B. McGee, D. R. Johnson, F. P. Downes, D. N. Cameron, N. H. Bean, and P. M. Griffin.** 1991. An outbreak of shigellosis at an outdoor music festival. Am. J. Epidemiol. **133:**608–615.
20. **Lilly, H. A., and J. L. Lowbury.** 1978. Transient skin flora. Their removal by cleansing or disinfection in relation to their mode of deposition. J. Clin. Pathol. **31:**919–922.
21. **Luber, P., S. Brynestad, D. Topsch, K. Scherer, and E. Bartelt.** 2006. Quantification of *Campylobacter* species cross-contamination during handling of contaminated fresh chicken parts in kitchens. Appl. Environ. Microbiol. **72:**66–70.
22. **Medema, G. J., P. F. M. Teunis, A. H. Havelaar, and C. N. Haas.** 1996. Assessment of the dose-response relationship of *Campylobacter jejuni*. Int. J. Food Microbiol. **30:**101–111.
23. **Meengs, M. R., B. K. Giles, C. D. Chisholm, W. H. Cordell, and D. R. Nelson.** 1994. Hand washing frequency in an emergency department. J. Emerg. Nurs. **20:**183–188.
24. **Montville, R., Y. Chen, and D. W. Schaffner.** 2001. Glove barriers to bacterial cross-contamination between hands to food. J. Food Prot. **64:**845–849.
25. **Oosterom, J.** 1998. The importance of hygiene in modern society. Int. Biodeterior. Biodegradation **41:**185–189.
26. **Paulson, D. S.** 1994. A comparative evaluation of five surgical hand scrub preparations. AORN J. **60:**246, 249–256.
27. **Quraishi, Z. A., M. McGuckin, and F. X. Blais.** 1984. Duration of handwashing in intensive care units: a descriptive study. Am. J. Infect. Control **12:**83–87.
28. **Rotter, M. L., and W. Koller.** 1992. Test models for hygienic handrub and hygienic handwash: the effects of two different contamination and sampling techniques. J. Hosp. Infect. **20:**163–171.
29. **Rubin, R. H., and L. Weinstein.** 1977. Salmonellosis: microbiologic, pathologic and clinical features. Stratton Intercontinental Medical Book Corp., New York, NY.
30. **Sandora, T. J., E. M. Taveras, M. C. Shih, E. A. Resnick, G. M. Lee, D. Ross-Degnan, and D. A. Goldmann.** 2005. A randomized controlled trial of a multifaceted intervention including alchohol-based hand sanitizer and hand-hygiene education to reduce illness transmission in the home. Pediatrics **116:**587–594.
31. **Schaffner, D. W., and K. M. Schaffner.** 2007. Management of risk of microbial cross-contamination from uncooked frozen hamburgers by alcohol-based hand sanitizer. J. Food Prot. **70:**109–113.
32. **Sickbert-Bennett, E. E., D. J. Weber, M. F. Gergen-Teague, and W. A. Rutala.** 2004. The effects of test variables on the efficacy of hand hygiene agents. Am. J. Infect. Control **32:**69–83.
33. **Soap and Detergent Association.** 2007. Clean hands report card. http://www.cleaning101.com./newsroom/2007_survey/keyFindings.cfm?retType=News.
34. **Strachan, N. J. C., M. P. Doyle, F. Kasuga, O. Rotariu, and I. D. Ogden.** 2005. Dose response modelling of *Escherichia coli* O157 incorporating data from foodborne and environmental outbreaks. Int. J. Food Microbiol. **103:** 35–47.
35. **Swanson, K. M. J., F. F. Busta, E. H. Peterson, and M. G. Johnson.** 1992. Colony count method, p. 75–95. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compedium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, DC.
36. **U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition.** 2007. Bad bug book: introduction to foodborne pathogenic microorganisms and natural toxins. U.S. Food and Drug Administration, Rockville, MD. http://www.cfsan.fda.gov/~mow/intro.html.
37. **World Health Organization.** 2005. WHO guidelines on hand hygiene in health care (advanced draft): a summary. World Health Organization, Geneva, Switzerland. http://www.who.int/patientsafety/events/05/HH_en.pdf.
38. **Zhao, P., T. Zhao, M. P. Doyle, J. R. Rubino, and J. Meng.** 1998. Development of a model for evaluation of microbial cross-contamination in the kitchen. J. Food Prot. **61:**960–963.