Applied and Environmental Microbiology, May 2011, p. 2898–2904 Vol. 77, No. 9
0099-2240/11/$12.00 doi:10.1128/AEM.02632-10

Bacterial Hand Contamination and Transfer after Use of Contaminated Bulk-Soap-Refillable Dispensers▽†

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Received 11 November 2010/Accepted 26 February 2011

Bulk-soap-refillable dispensers are prone to extrinsic bacterial contamination, and recent studies demonstrated that approximately one in four dispensers in public restrooms are contaminated. The purpose of this study was to quantify bacterial hand contamination and transfer after use of contaminated soap under controlled laboratory and in-use conditions in a community setting. Under laboratory conditions using liquid soap experimentally contaminated with 7.51 $\log_{10}$ CFU/ml of *Serratia marcescens*, an average of 5.28 $\log_{10}$ CFU remained on each hand after washing, and 2.23 $\log_{10}$ CFU was transferred to an agar surface. In an elementary-school-based field study, Gram-negative bacteria on the hands of students and staff increased by 1.42 $\log_{10}$ CFU per hand (26-fold) after washing with soap from contaminated bulk-soap-refillable dispensers. In contrast, washing with soap from dispensers with sealed refills significantly reduced bacteria on hands by 0.30 $\log_{10}$ CFU per hand (2-fold). Additionally, the mean number of Gram-negative bacteria transferred to surfaces after washing with soap from dispensers with sealed-soap refills (0.06 $\log_{10}$ CFU) was significantly lower than the mean number after washing with contaminated bulk-soap-refillable dispensers (0.74 $\log_{10}$ CFU; $P < 0.01$). Finally, significantly higher levels of Gram-negative bacteria were recovered from students (2.82 $\log_{10}$ CFU per hand) than were recovered from staff (2.22 $\log_{10}$ CFU per hand) after washing with contaminated bulk soap ($P < 0.01$). These results demonstrate that washing with contaminated soap from bulk-soap-refillable dispensers can increase the number of opportunistic pathogens on the hands and may play a role in the transmission of bacteria in public settings.

Hand washing with soap and water is a universally accepted practice for reducing the transmission of potentially pathogenic microorganisms. However, liquid soap can become contaminated with bacteria and poses a recognized health risk in health care settings. In particular, bulk-soap-refillable dispensers (ones in which new soap is poured into a dispenser) are prone to bacterial contamination, and several outbreaks linked to the use of contaminated soap in health care settings have been reported (2, 3, 5, 15, 18, 22–24). The Centers for Disease Control and Prevention (CDC) "Guideline for Hand Hygiene in Health-Care Settings" addresses this risk in a recommendation: "Do not add soap to a partially empty soap dispenser. This practice of 'topping off' dispensers can lead to bacterial contamination of soap" (4). This "category IA recommendation" was "strongly supported by well-designed experimental, clinical, and epidemiologic studies." (4) Sealed-soap-dispensing systems, in contrast, are typically refilled by inserting into the dispenser a new bag or cartridge of soap that usually includes a new nozzle.

Bulk-soap-refillable dispensers are the predominant dispenser type in community settings, such as public restrooms. However, few studies have been conducted to evaluate the occurrence of microbial soap contamination in community settings. One study, conducted in Japan, examined bacterial contamination of hand washing soaps obtained from restrooms of various public use facilities. The authors found 17 different species of bacteria, many of which were opportunistic pathogens, including *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter* species, and *Pseudomonas* species (1). Recent studies conducted in the United States demonstrated that 25% of bulk-soap-refillable dispensers in public restrooms were excessively contaminated (8). Bacterial loads averaged more than 10^6 CFU/ml of soap, and 16% of the samples contained coliform bacteria. Interestingly, of the 15 different species isolated in this study, 7 were identical to those found in the Japanese study, including both *K. pneumoniae* and *S. marcescens.* Both *S. marcescens* and *K. pneumoniae* are opportunistic pathogens known to transmit via the hands (7, 17, 21).

Despite these findings, the public health risk associated with the use of contaminated bulk-soap-refillable dispensers in community settings is unclear. It would be very difficult if not impossible to trace the source of a community-acquired infection back to contaminated soap in a public restroom. Therefore, to better understand this risk, a greater understanding of the potential for bacteria from contaminated soap to remain on the hands and to be transferred to secondary surfaces after washing with contaminated soap is needed. The objectives of this study were to (i) quantify the levels of bacteria remaining on hands after washing with contaminated soap; (ii) quantify the transfer of contaminating bacteria from the hands to a secondary surface; and (iii) collect microbiological data in a field setting under actual use conditions. To our knowledge this is the first study of its kind in any setting.

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▽ Published ahead of print on 18 March 2011.

† The authors have paid a fee to allow immediate free access to this article.

MATERIALS AND METHODS

Controlled hand washing studies. (i) Test articles. The liquid test soap contained a surfactant system representative of soaps found in public restrooms but did not contain preservatives. Soap was prepared by mixing 1,648 g of soft water, 17 g of ammonium chloride, 330 g of surfactant blends (Lubrizol Advanced Materials, Cleveland, OH; and Rhodia, Inc., Mississauga, Ontario, Canada), 2 g of fragrance (Flavorchem Orchidia, Downers Grove, IL), and 2 g of citric acid. Contaminated-soap samples were prepared by 8 successive inoculations with 300-μl to 10-ml aliquots of overnight tryptic soy broth (TSB) cultures of the marker organism *S. marcescens* ATCC 14756 or *K. pneumoniae* ATCC 13883 over the 3-week period prior to the hand wash test date. Populations of marker organisms were determined by standard plating on tryptic soy agar (TSA) and monitored over time to achieve the target contamination level.

(ii) Subjects. Eighteen subjects participated in controlled study I, and 16 participated in study II. Subjects recruited from the Bozeman, MT, area were at least 18 years of age, and the study demographics were mixed for age, sex, and race. Exclusion criteria included dermatoses or other injuries to the skin of the hands or forearms or any other conditions that would have compromised the subjects and the study.

(iii) Study design. Two controlled studies (study I and study II) assessed bacterial hand contamination and transfer post-hand washing with contaminated or uncontaminated soap. Protocols were approved by the Gallatin Institutional Review Board (Bozeman, MT). In study I, 6 subjects washed with uncontaminated test soap, 6 subjects washed with soap contaminated with *K. pneumoniae* (5.85 $\log_{10}$ CFU/ml), and 6 subjects washed with soap contaminated with *S. marcescens* (3.72 $\log_{10}$ CFU/ml). Following the hand wash, hands were sampled for residual *S. marcescens* and/or *K. pneumoniae* as described below. In study II, 8 subjects washed with soap contaminated with a low level of *S. marcescens* (4.51 $\log_{10}$ CFU/ml), and 8 subjects washed with soap contaminated with a high level of *S. marcescens* (7.51 $\log_{10}$ CFU/ml). Following the hand wash, the hands of 6 random subjects per test soap were sampled for residual *S. marcescens*. Two subjects per test soap touched agar plates to create hand imprints of bacteria transferred to the agar surfaces.

(iv) Hand washing procedure. Water used for wetting and rinsing the hands was maintained at a temperature of 40°C ± 2°C. In study I, subjects washed with 5 ml of test soap for 30 s, followed by a 30-s water rinse. In study II, subjects washed with 1.5 ml of test soap for 10 s followed by a 10-s rinse.

(v) Bacterial recovery and enumeration. To recover bacteria from the hands, powder-free, sterile, latex gloves were placed on subjects' hands, 75 ml of a recovery solution (0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, and 1.0 g isooctylphenoxypolyethoxyethanol [Triton X-100] in 1 liter distilled water [pH adjusted to 7.8]) was transferred into each glove, and gloves were secured above the wrist. Technicians massaged the hands through the gloves for 60 s. Within 1 min of completing the massage, a 5-ml aliquot of the "glove juice" sample was removed and serially diluted in Butterfield's phosphate buffer solution containing lecithin and polysorbate 80 (BPB+). Dilutions were plated in duplicate onto appropriate agar plates by spread plating 1.0 ml of the recovery solution manually and spiral plating 50-μl aliquots of all dilutions (Spiral Biotech Autoplate; Advanced Instruments, Inc., Norwood, MA). *S. marcescens* was recovered on TSA with lecithin and polysorbate 80 (TSA+) and incubated for 24 to 48 h at 25°C. *K. pneumoniae* was recovered on MacConkey agar and incubated for 24 to 48 h at 35°C (7). Colonies with a morphology qualitatively similar to that of the marker organism were counted (i.e., red pigment on TSA+ for *S. marcescens* and pink mucoid on MacConkey agar for *K. pneumoniae*) with a plate-counting system (QCount model 510; Advanced Instruments, Inc., Norwood, MA). For the hand-stamp sampling procedure, subjects pressed the palms of the hands onto TSA+-containing polystyrene bioassay trays for 15 s. Trays were placed in laminar flow hoods to remove residual moisture and then incubated for 24 to 48 h at 25°C.

Field hand washing study. (i) Study site and test site. The field study was conducted in the restrooms of an elementary school in Ohio. Twenty-two subjects participated, including 12 adult staff members (teachers, administration, and janitorial staff) and 10 students (fourth and fifth grades). Exclusion criteria included cuts, rashes, or other skin conditions that would have compromised the subjects and the study. All adult subjects signed an informed consent form preapproved by Chesapeake Research Review, Inc. (Columbia, MD). All students participated only after signed parental consent, which was also preapproved by Chesapeake Research Review, Inc.

(ii) Test articles and assessment of microbial contamination. The contaminated soap used in the field study was a commercially available antimicrobial soap formulation that had been in use in the school for years prior to this study. Samples were obtained from all 14 bulk-soap-refillable soap dispensers used in the school restrooms. Approximately 10 ml of soap was aseptically collected from the dispenser nozzle into sterile 50-ml conical centrifuge tubes. The sealed-soap dispensers contained a foam soap which was sampled by filling a 120-ml sterile cup with foam. Samples were vortexed for at least 30 s and placed at rest until all bubbles dissipated. An aliquot of soap was removed with a positive displacement pipette and serially diluted in BPB+. One hundred microliters of each dilution was spiral plated onto R2A agar plates in duplicate. R2A agar is a nonselective medium designed for heterotrophic plate counts from potable water and has been previously used to quantify levels of bacteria in contaminated soap (8). Plates were incubated for 96 h at 37°C, and colonies were enumerated by hand by the standard spiral plate count methodology. The number of CFU/ml of bacteria in the original soap sample was determined based on the average colony count and the dilution factor. Soaps were considered to be contaminated if the level exceeded 1,000 CFU/ml, which is the level typically considered acceptable in nonsterile cosmetic products (13). Representatives of each dominant colony type were streak purified by multiple passages on TSA. Bacterial species were identified by using AP120E strips (bioMérieux, Marcy-l'Etoile, France). Contamination levels were monitored in the bulk-soap dispensers for 3 months prior to the hand washing trials (data not shown). All soap samples used in hand washing trials were also collected and tested for the presence of contaminating bacteria on the same days that the hand washing trials were conducted.

(iii) Study design. The study protocol was approved by Chesapeake Research Review, Inc., and was conducted in compliance with procedures approved under this protocol. Hand wash trials were conducted in 14 different restroom locations throughout the school. Technicians executing the study were of the gender indicated by the restroom. In phase I of the study, the contaminated bulk soap and uncontaminated bulk soap trials were conducted over a 4-day period. The bulk dispensers were then replaced with sealed-system dispensers. Phase II of the study, which evaluated the sealed system, was conducted 6 months later and was completed in 1 day. Each subject participated in up to 6 hand washes total for the entire study. No subject participated in more than 2 hand washes on a single day, and a minimum of 30 min was required between each hand wash. Each subject's visit consisted of a pre-hand wash (baseline) sampling, a hand wash, and a post-hand wash sampling. Right and left hands were randomized for glove juice or hand-stamp sampling at the first wash for each participant and alternated at each subsequent wash.

(iv) Hand washing and decontamination procedures. Subjects were asked to wash their hands with soap as they normally would do when washing after using the restroom facilities. The amount of soap and the length and technique of washing, rinsing, and towel drying were at the discretion of each test subject. The temperature of the water used was not controlled. The participants' hands were decontaminated at the end of each visit by washing with soap from a bottle of commercially available uncontaminated soap and then sanitizing with an ethanol-based hand sanitizer.

(v) Bacterial recovery and enumeration. The glove juice sampling method was performed similarly to the controlled study method, except for a few modifications designed to improve the detection limit of the method. Fifty milliliters of recovery solution was added to each glove, and all of the solution recovered from each hand sample was transferred to a sterile 50-ml centrifuge tube. The solution was centrifuged (10 min at 5,000 × *g*) to concentrate the bacteria. Pilot testing verified the effectiveness of the concentration method. All but 5 ml of recovery solution supernatant was removed, and the pellet was vortexed for 1 min to resuspend the cells back into the remaining 5 ml. One milliliter of the concentrated recovery solution was pour plated in duplicate, and 0.1 ml of 10-fold dilutions prepared in BPB+ was spiral plated. All plating was conducted in duplicate on both MacConkey and Chromagar orientation agar (BD, Franklin Lakes, NJ). MacConkey agar was used to select for Gram-negative bacteria. Chromagar orientation results are not presented here, but were used to qualitatively verify that MacConkey plates were adequately selective for contaminants in the soap (versus normal skin microbiota). For the hand-stamp method, subjects placed the palms of their hands and fingers onto MacConkey agar plates for 10 s. All agar plates were incubated for 96 h at 37°C and photographed for archiving. Colonies present on the MacConkey plates were counted.

Data analysis and statistical considerations. For the controlled studies, the estimated $\log_{10}$ number of viable *S. marcescens* or *K. pneumoniae* cells recovered from each hand (the "*R* value") was determined with the formula $R = \log_{10}(75 \times C_i \times 10^D)$, where 75 is the amount (ml) of recovery solution instilled in each glove, C_i is the arithmetic average colony count of the 2 plate counts at a particular dilution, and D is dilution factor. The limit of detection for the controlled studies was 1.57 $\log^{10}$ CFU/hand. For the field study, the total number of Gram-negative bacteria recovered from each hand was determined by the

TABLE 1. Bacteria recovered from hands after washing with contaminated liquid soap

Bacterial contaminant (marker organism)	Bacterial load		Postwash bacterial recovery (mean $\log_{10}$ CFU/hand ± SD [n = 12])
	Test soap ($\log_{10}$ CFU/ml)	Applied ($\log_{10}$ CFU/hand)	
None	0.00	0.00	<1.57[a]
Klebsiella pneumoniae	5.85	6.25	2.74 ± 0.5
Serratia marcescens	3.72	4.12	3.60 ± 0.2[b]

[a] Limit of detection.
[b] Greater bacterial recovery per hand after washing with soap contaminated with *Serratia marcescens* versus washing with soap contaminated with *Klebsiella pneumoniae.* $P < 0.0001$ by unpaired two-sample t test.

TABLE 2. Bacteria recovered and transferred from hands after washing with soap contaminated with *S. marcescens*

Bacterial load in test soap ($\log_{10}$ CFU/ml)	Bacterial load applied ($\log_{10}$ CFU/hand)	Postwash bacterial recovery (n = 12)		Postwash bacterial transfer (n = 4)	
		Mean $\log_{10}$ CFU/hand ± SD	P value	Mean $\log_{10}$ CFU/hand ± SD	P value
4.51	4.39	1.70 ± 0.27		0.30 ± 0.42	
7.51	7.39	5.28 ± 0.47	<0.0001[a]	2.23 ± 0.49	0.001[a]

[a] Unpaired two-sample t test.

formula $R = \log_{10}(5 \times C_i \times 10^D)$. The limit of detection for the field studies was 0.40 $\log_{10}$ CFU/hand. The total numbers of bacteria transferred to MacConkey agar hand-stamp plates were counted directly from the agar plates. Results were obtained by analysis of 91 hand wash trials that yielded usable results for all four measurements taken (CFU recovered before, CFU recovered after, CFU transferred before, and CFU transferred after). Raw CFU values were converted to $\log_{10}$ CFU values, and statistical comparisons were performed by using paired and unpaired t tests on GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, CA).

RESULTS

Recovery and transfer of bacteria from hands after washing with experimentally contaminated liquid soap. Human subjects washed for 30 s with 5 ml of soap experimentally contaminated with either *K. pneumoniae* (5.85 $\log_{10}$ CFU/ml) or *S. marcescens* (3.72 $\log_{10}$ CFU/ml) followed by a 30-s rinse. Neither test organism was recovered from the hands of subjects prior to washing hands or from the subjects that washed with uncontaminated control soap. In contrast, for *K. pneumoniae*, a mean of 2.74 $\log_{10}$ CFU/hand was recovered from subjects after washing with *K. pneumoniae*-contaminated soap, and for *S. marcescens*, a mean of 3.60 $\log_{10}$ CFU/hand was recovered from subjects after washing with *S. marcescens*-contaminated soap (Table 1). Interestingly, more bacteria were recovered from hands washed with *S. marcescens*-contaminated soap than from those washed with *K. pneumoniae*-contaminated soap ($P < 0.0001$), even though the level of *K. pneumoniae* contamination was 100-fold higher.

In a second experiment, subjects performed a 10-s hand wash with 1.5 ml of liquid soap experimentally contaminated with either a high level of *S. marcescens* (7.51 $\log_{10}$ CFU/ml) or with a low level of *S. marcescens* (4.51 $\log_{10}$ CFU/ml) followed by a 10-s rinse. It is known that when soap that is not contaminated is used for hand washing, it is more effective at removing transient bacteria when greater volumes of soap and longer wash times are used (11). Therefore, the second controlled study was conducted under conditions chosen to be more representative of the hand washing behaviors typically observed (6, 12, 14, 16, 19). The mean numbers of *S. marcescens* cells recovered after washing with high- and low-level-contaminated soap were 5.28 $\log_{10}$ CFU and 1.70 $\log_{10}$ CFU per hand, respectively (Table 2) ($P < 0.0001$). The number of bacteria transferred to an agar surface after washing were 2.23 $\log_{10}$ CFU and 0.30 $\log_{10}$ CFU per hand for the high- and low-level-contaminated soap, respectively (Table 2 and Fig. 1) ($P = 0.001$).

Recovery and transfer of bacteria from hands after washing with contaminated liquid soap in an elementary school. An elementary school was identified in which all (14/14) of the bulk-soap-refillable dispensers being used in the restrooms were found to be contaminated with bacteria at levels ranging from 6.0 to 7.0 $\log_{10}$ CFU/ml of soap (Table 3). A variety of Gram-negative species from the *Citrobacter*, *Providencia*, *Pseudomonas*, and *Serratia* genera were identified among the recovered contaminants. All of the contaminated dispensers were replaced with sealed-soap-dispensing systems after the first phase of the field hand washing study. After 1 year postinstallation, all of the soap dispensed from the sealed-soap dispensers was confirmed to be contamination free.

A study was conducted with students and staff to assess the levels of Gram-negative bacteria remaining on or transferred from hands after washing with contaminated soap from these dispensers or with uncontaminated control soaps (Table 4). Prior to washing with contaminated bulk soap, uncontaminated bulk soap, and uncontaminated soap from sealed refills, the mean numbers of bacteria recovered from hands of subjects were 1.17, 0.99, and 1.67 $\log_{10}$ CFU per hand, respectively. The mean number of bacteria recovered from the hands after hand washing with the contaminated soap (2.59 $\log_{10}$ CFU per hand) was significantly higher than the pre-hand-washing value ($P < 0.0001$). Gram-negative bacteria were detected in 97% (60/62) of hands tested after washing with bulk soap compared to 52% (32/62) before washing. In contrast, the mean number of bacteria recovered from hands after washing with uncontaminated bulk soap (0.82 $\log_{10}$ CFU per hand) was reduced compared to the prewashing numbers. When hands were washed with uncontaminated soap from the new replacement sealed-system dispensers, the mean numbers of bacteria recovered from hands after washing (1.37 $\log_{10}$ CFU per hand) were also reduced compared to the prewashing numbers and were statistically lower than those recovered from hands washed with contaminated soap ($P < 0.0001$). The mean number of Gram-negative bacteria recovered from the hands after washing with contaminated soap was significantly higher for students (2.82 $\log_{10}$ CFU per hand) than that for staff (2.22 $\log_{10}$ CFU per hand; $P = 0.008$) (Table 5).

Figure 2 compiles $\log_{10}$ CFU changes after individual hand washes into a histogram in which the bars represent the number of times each reduction or increase was observed. When contaminated soap was used, an increase was observed for 55 of 62 hand washes (89%), and the mean change was a 1.42-$\log_{10}$ CFU increase. In contrast, when uncontaminated soap (bulk or sealed) was used, an increase was observed for only 3

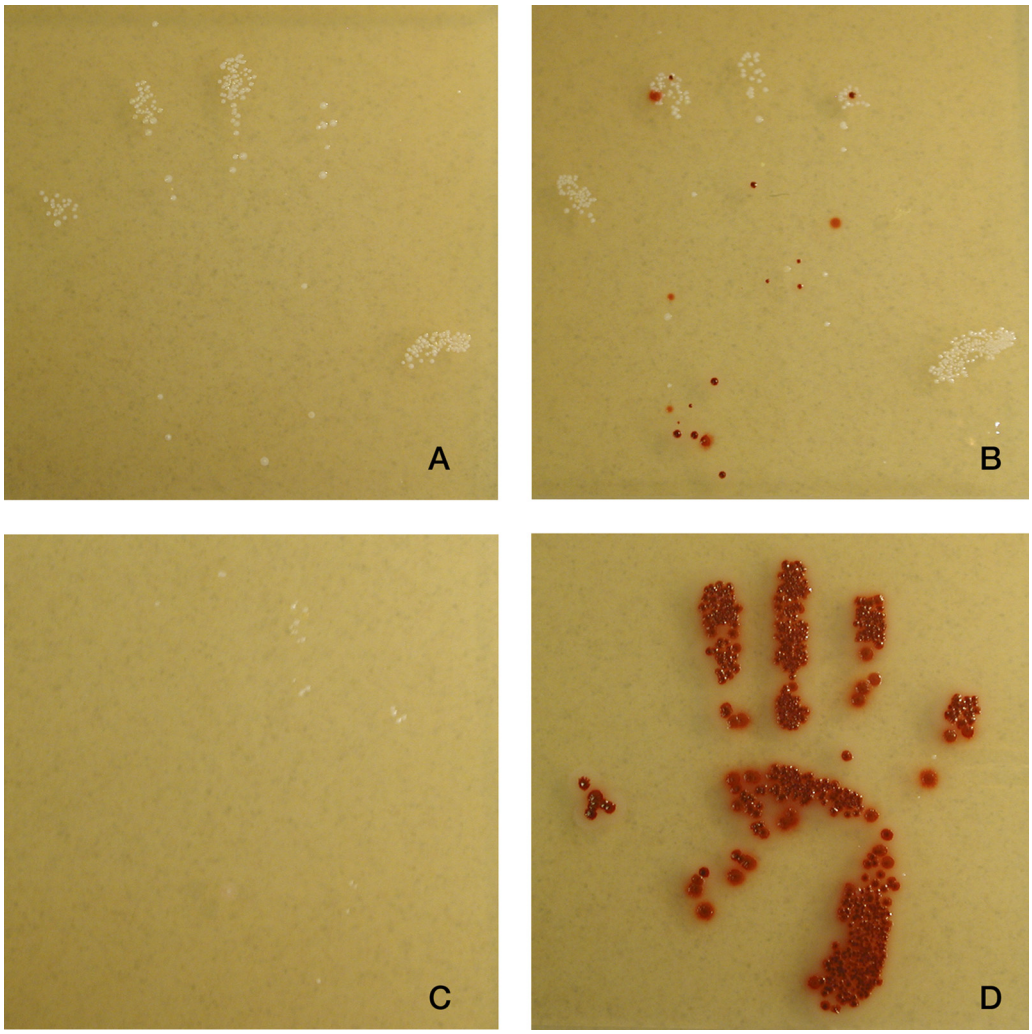

FIG. 1. Sample images from a controlled study (Table 2) to determine the number of bacteria from contaminated hands transferred to an agar surface before (A and C) and after (B and D) hand washing with soap containing 4.51 $\log_{10}$ CFU/ml (A and B) or 7.51 $\log_{10}$ CFU/ml (C and D) of *S. marcescens*.

of 29 hand washes (10%), and the mean change was a 0.26-$\log_{10}$ CFU decrease.

Prior to washing, subjects transferred on average 0.10, 0.10, and 0.18 $\log_{10}$ CFU/hand of Gram-negative bacteria to touched agar surfaces. This number increased significantly after washing with soap from the contaminated dispensers (0.74 $\log_{10}$ CFU/hand; $P < 0.0001$) (Table 4). Washing with uncontaminated-soap controls did not significantly change the mean number of transferred Gram-negative bacteria ($P = 0.945$, uncontaminated bulk soap; $P = 0.100$, uncontaminated sealed soap). Furthermore, fewer bacteria were transferred from subjects' hands after washing with uncontaminated sealed soap (0.06 versus 0.74 $\log_{10}$ CFU; $P = 0.0004$) or uncontaminated bulk soap (0.09 versus 0.74 $\log_{10}$ CFU; $P = 0.012$), compared to bacteria that were transferred from subjects' hands after washing with contaminated soap. Transfer of at least 1 CFU of Gram-negative bacteria after washing was observed in 61% (38/62) of hands washed with contaminated soap versus 21% (4/19) of hands washed with uncontaminated sealed soap. In addition, significantly more Gram-negative bacteria were transferred to agar surfaces touched by students (0.98 $\log_{10}$ CFU per hand) after using contaminated soap than by the adult staff (0.37 $\log_{10}$ CFU per hand; $P = 0.003$) (Table 5).

A comparison of the pre- and postwash recoveries of bacteria for the individual bulk-soap-refillable dispensers tested in the field study is shown in Fig. 3. The number of bacteria recovered from hands postwash increased significantly relative to the prewash recoveries for all of the contaminated dispensers, and the increase was significant for 8 of the 14 contaminated dispensers (P values ranging from 0.0003 to 0.03). In contrast, the number of bacteria recovered from hands after washing with the uncontaminated control soaps decreased relative to the prewash recoveries, but was not significant ($P = 0.199$ for control 1, and $P = 0.324$ for control 2).

DISCUSSION

The purpose of hand washing is to remove soil and to reduce the level of potentially pathogenic transient microorganisms. This is the first study to quantitatively demonstrate that wash-

TABLE 3. Identification of bacteria isolated from bulk-soap-refillable soap dispensers in an elementary school

Soap dispenser tested	Total bacterial contamination in soap (log_{10} CFU/ml)	Presence of:							
		Unknown species	*Citrobacter freundii*	*Citrobacter youngae*	*Providencia rettgeri*	*Providencia stuartii*	*Pseudomonas aeruginosa*	*Pseudomonas fluorescens*	*Serratia rubidaea*
1	6.9				+				+
2	6.8				+				
3	6.3		+				+		
4	6.4				+		+		
5	6.0		+				+		
6	6.4		+			+			
7	6.0						+		
8	6.7			+	+				
9	6.2				+				
10	6.2		+		+				
11	6.4						+		
12	6.2	+						+	
13	7.0				+		+		
14	6.0		+		+				

ing hands with contaminated liquid soap actually increases the number of Gram-negative bacteria on hands. Furthermore, the results directly demonstrate that bacteria from contaminated hands can be transferred to secondary surfaces. We therefore conclude that washing with contaminated soap not only defeats the purpose of hand washing but may contribute to the transmission of potentially harmful bacteria. The results of the two laboratory hand washing studies were corroborated by the elementary school field study, which demonstrated a 26-fold increase in the number of Gram-negative bacteria present on the hands (Table 4) after washing with contaminated soap from bulk-soap-refillable dispensers, demonstrating a potential public health risk in public, non-health-care settings. Importantly, when the contaminated dispensers in the school were replaced with dispensers containing sealed-soap refills, none were found to be contaminated after 12 months of use. Furthermore, washing hands with soap from the sealed-soap system reduced the number of bacteria on hands of the study participants (Table 4). Taken together, these results indicate that use of dispensers with sealed refills instead of open bulk-soap-refillable dispensers can lower the risk of extrinsic microbial contamination and can reduce the spread of potentially pathogenic bacteria.

Previous studies have demonstrated an association between the use of bulk-soap-refillable dispensers and bacterial contamination of the liquid soap. Contamination rates in these studies ranged from 20% to 25% (8; C. A. Zapka, M. Chattman, S. L. Maxwell, D. R. Macinga, M. J. Dolan, and C. P. Gerba, unpublished data). In the present study, we found that 100% (Table 3) of bulk soap dispensers in one elementary school were contaminated. A single soap formulation was used in the school and was dispensed from two similar bulk-soap dispensers. In previous studies, multiple sites using different soap formulations and different dispensers were surveyed. These differences may account for the higher rate of contamination in this facility. Further analysis of the factors contributing to the unusually high prevalence of contaminated soap in this school will be presented elsewhere (C. A. Zapka, unpublished data). Many of the bacteria isolated from the bulk soap in the elementary school are considered to be opportunistic pathogens and can cause infections in compromised populations (10, 15). In fact, use of a shampoo contaminated with *Pseudomonas aeruginosa*, an organism found in 43% (6/14) of the dispensers in this elementary school (Table 3), has been reported to have led to a fatality (9).

The levels of bacteria in the soaps tested in the two laboratory hand washing studies (3.72 to 7.51 log_{10} CFU/ml) were representative of those encountered in this and our previous field studies (2.77 to 7.81 log_{10} CFU/ml) (8; C.A. Zapka, unpublished data). Significantly higher levels of *S. marcescens* were recovered from the hands despite a lower level of contamination in the test soap compared to *K. pneumoniae* (Table 1). These results suggest that the two organisms may interact with human skin in qualitatively different ways. Both organisms have been reported to contaminate soaps and lead to infections in health care settings (5, 18, 20, 22). Even a brief contact

TABLE 4. Gram-negative bacteria recovered and transferred from the hands of students and staff in an elementary school before and after hand washing

Test soap type	No. of hand washes	Bacteria recovered/hand			Bacteria transferred/hand		
		Mean log_{10} CFU ± SD		*P* value[a]	Mean log_{10} CFU ± SD		*P* value[a]
		Before hand wash	After hand wash		Before hand wash	After hand wash	
Contaminated bulk soap	62	1.17 ± 0.70	2.59 ± 0.89	<0.0001	0.10 ± 0.31	0.74 ± 0.81	<0.0001
Uncontaminated bulk soap	10	0.99 ± 0.39	0.82 ± 0.19	0.084	0.10 ± 0.32	0.09 ± 0.28	0.945
Uncontaminated sealed soap	19	1.67 ± 0.98	1.37 ± 0.81	0.025	0.18 ± 0.37	0.06 ± 0.20	0.100

[a] Log_{10} CFU before versus after by paired two-sample *t* test.

TABLE 5. Influence of gender and age on Gram-negative bacteria recovered and transferred from hands washed with contaminated bulk soap in an elementary school

Participant type	No. of hand washes	Bacteria recovered/hand				Bacteria transferred/hand			
		Before hand wash (mean $\log_{10}$ CFU ± SD)	*P* value[a]	After hand wash (mean $\log_{10}$ CFU ± SD)	*P* value[a]	Before hand wash (mean $\log_{10}$ CFU ± SD)	*P* value[a]	After hand wash (mean $\log_{10}$ CFU ± SD)	*P* value[a]
Students									
Male	19	0.95 ± 0.52	0.222	2.49 ± 1.01	0.024	0.10 ± 0.37	0.575	0.71 ± 0.89	0.047
Female	19	1.22 ± 0.78		3.15 ± 0.69		0.17 ± 0.37		1.25 ± 0.71	
Staff									
Male	13	1.53 ± 0.91	0.134	2.37 ± 0.72	0.253	0.08 ± 0.23	0.450	0.42 ± 0.61	0.688
Female	11	1.06 ± 0.44		2.03 ± 0.70		0.03 ± 0.09		0.32 ± 0.61	
All									
Students	38	1.09 ± 0.66	0.218	2.82 ± 0.91	0.008	0.13 ± 0.37	0.344	0.98 ± 0.84	0.003
Staff	24	1.31 ± 0.76		2.22 ± 0.71		0.06 ± 0.18		0.37 ± 0.60	

[a] Male versus female or students versus staff by unpaired two-sample *t* test.

(10 s) with contaminated soap resulted in detectable levels of bacteria on hands (Table 2). Significantly higher levels of *S. marcescens* were recovered from the hands and were transferrable to a secondary surface when the liquid soap was contaminated with a higher bacterial load. These results demonstrate that both the identity of the microbial contaminant and the level of contamination are important factors influencing the public health risk associated with the use of contaminated soap.

The elementary school field study revealed that students retained more bacteria on the hands and transferred significantly more after washing with contaminated bulk soap than the adult staff (Table 4). Although the reasons for these observed differences are not clear, we hypothesize that differences in hand size, skin condition, and/or hand washing technique (e.g., thoroughness of water rinsing and paper towel drying) may be contributing factors. Children represent a vulnerable population with potentially a greater susceptibility to bacterial infections due to their less developed immune systems. Hence, further studies to identify these factors are warranted.

The number of bacteria transferred to agar surfaces was directly proportional to the number of bacteria recovered from subjects' hands post-hand washing in both laboratory studies and in the field study. Analysis of the combined data set showed the concentration of bacteria in contaminated soap correlated positively with both the number of CFU recovered from the hands ($P < 0.0001$) and the number of CFU transferred from the hands ($P < 0.0001$) post-hand washing (data not shown). Based on the observed correlations, washing with soap containing less than 3.7 $\log_{10}$ CFU of bacteria/ml would not lead to detectable bacteria on the hands, and washing with soap with less than 5.4 $\log_{10}$ CFU/ml would not result in detectable transfer of the bacteria to touched surfaces. Coincidentally, this observation confirms the appropriateness of a current industry guideline that recommends that cosmetic products contain less than 3.0 $\log_{10}$ CFU of bacteria/g (13).

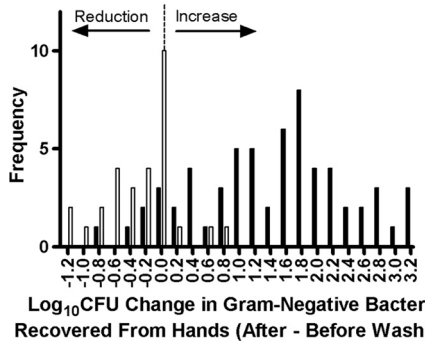

FIG. 2. $\log_{10}$ CFU change in Gram-negative bacteria recovered from hands of elementary school students and staff as a result of hand washing with contaminated soap (solid bars) versus uncontaminated control soaps (open bars).

In summary, this study is the first to quantify the levels of bacteria remaining on hands after washing with contaminated soap and to quantify the transfer of contaminating bacteria from the hands to a secondary surface. This research confirms previous work demonstrating a strong association between open bulk-soap-refillable soap dispensers and extrinsic bacterial soap contamination and demonstrates that washing with contaminated soap poses a potential public health risk in community settings. Our findings further show that extrinsic contamination of hand soap can be eliminated or considerably reduced through the use of sealed-soap-dispensing systems.

Limitations of our study that future studies should be designed to address include species identification of the entire microbial communities present on the hands before and after washing, comparison of results between dominant and nondominant hands, and correlation of hand washing techniques (volume of soap used, length of washing and rinsing, paper towel use behaviors, etc.) employed by participants with the observed results. Further studies to confirm these preliminary findings and to develop accurate risk models should be considered. Epidemiological studies of the causal relationship between contaminated soap and disease would be very useful to quantify the risk; however, they may be impractical to execute. The lack of such study data, however, should not preclude proactive efforts to reduce the

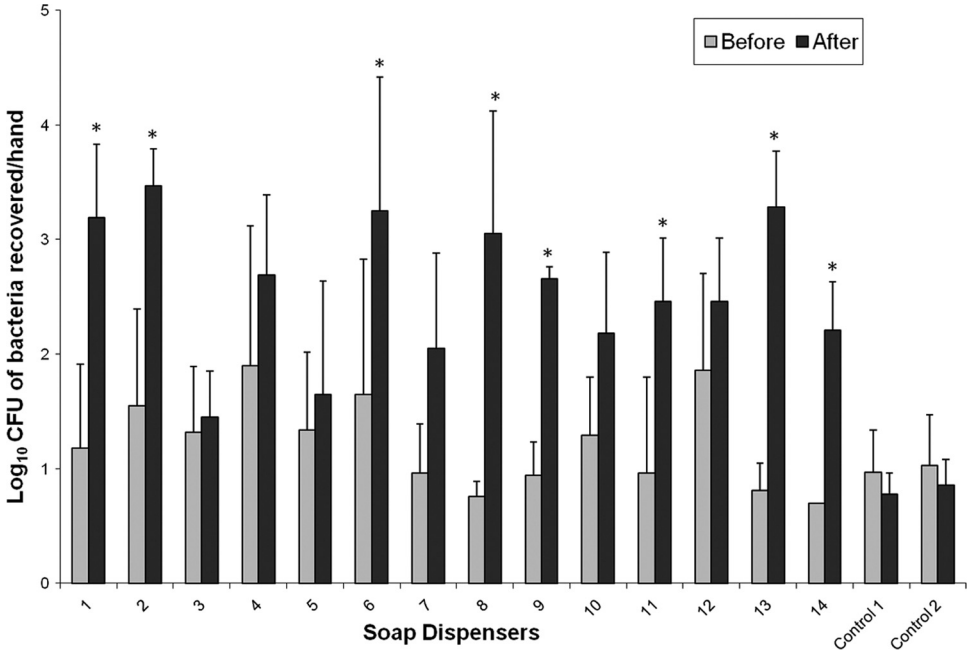

FIG. 3. Gram-negative bacteria recovered from the hands of elementary school students and staff before and after washing with contaminated bulk soap. Fourteen contaminated soap dispensers and 2 uncontaminated soap controls were used by students and staff. $n = 4$ for dispensers 2 to 5 and 12, and $n = 2$ for dispenser 10; $n = 5$ for all other dispensers. *, $P < 0.05$ for bacteria recovered per hand before versus after hand washing by paired two-sample t test.

unnecessary public health risks posed by open bulk-soap-refillable dispensers.

ACKNOWLEDGMENTS

We thank Lakshmi Kamath for assistance with the preparation of the manuscript and John Mitchell for thoughtful review of multiple drafts.

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