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Research Paper

Influence of Soap Characteristics and Food Service Facility Type on the Degree of Bacterial Contamination of Open, Refillable Bulk Soaps

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ABSTRACT

Concern has been raised regarding the public health risks from refillable bulk-soap dispensers because they provide an environment for potentially pathogenic bacteria to grow. This study surveyed the microbial quality of open refillable bulk soap in four different food establishment types in three states. Two hundred ninety-six samples of bulk soap were collected from food service establishments in Arizona, New Jersey, and Ohio. Samples were tested for total heterotrophic viable bacteria, *Pseudomonas,* coliforms and *Escherichia coli,* and *Salmonella*. Bacteria were screened for antibiotic resistance. The pH, solids content, and water activity of all soap samples were measured. Samples were assayed for the presence of the common antibacterial agents triclosan and parachlorometaxylenol. More than 85% of the soap samples tested contained no detectable microorganisms, but when a sample contained any detectable microorganisms, it was most likely contaminated at a very high level (~7 log CFU/mL). Microorganisms detected in contaminated soap included *Klebsiella oxytoca, Serratia liquefaciens, Shigella sonnei, Enterobacter gergoviae, Serratia odorifera,* and *Enterobacter cloacae*. Twenty-three samples contained antibiotic-resistant organisms, some of which were resistant to two or more antibiotics. Every sample containing less than 4% solids had some detectable level of bacteria, whereas no samples with greater than 14% solids had detectable bacteria. This finding suggests the use of dilution and/or low-cost formulations as a cause of bacterial growth. There was a statistically significant difference ($P = 0.0035$) between the fraction of bacteria-positive samples with no detected antimicrobial agent (17%) and those containing an antimicrobial agent (7%). Fast food operations and grocery stores were more likely to have detectable bacteria in bulk-soap samples compared with convenience stores ($P < 0.05$). Our findings underscore the risk to public health from use of refillable bulk-soap dispensers in food service establishments.

Key words: Bulk soap; Coliforms; Contamination; Hand washing

Washing hands with soap and water is a universally accepted practice to reduce cross-contamination and the incidence of nosocomial infections *(9, 12–14, 16, 18, 20, 26, 29, 33)*. The U.S. Food and Drug Administration (FDA), the U.S. Centers for Disease Control and Prevention (CDC), and the World Health Organization (WHO) suggest proper hand hygiene with soap and water and/or an alcohol-based hand sanitizer in health care and food preparation settings *(3, 35, 39)*. The CDC and WHO recommend alcohol-based hand sanitizer as the primary means for hand hygiene at key moments in health care settings *(3, 31, 39),* whereas food handling guidance from FDA *(35)* supports gloving or hand washing for primary prevention. The respective hand hygiene guidance documents from these three public health agencies all have language that indicates that a hand wash is not complete without the use of soap *(3, 35, 39)*. However, concern has been raised that the use of refillable bulk-soap dispensers is a public health risk because they provide an environment for potentially pathogenic bacteria to grow, especially if the bulk soap is diluted with water to reduce cost *(8, 21, 25, 30, 40)*.

Outbreaks associated with contaminated soap have been extensively documented in health care settings *(1, 2, 5, 24, 27, 30, 38),* but none to date have been connected to food service settings. Organisms found in bulk soaps are primarily gram-negative bacteria *(8),* and these bacteria include microorganisms that are commonly associated with nosocomial infection in hospitals *(3, 19)*. *Klebsiella pneumonia,* a bacterium associated with contaminated bulk soaps, can cause community-acquired pneumonia; proper hand hygiene is a good way of preventing cross-contamination by these bacteria because health care workers' hands can be vectors for these organisms *(7)*. Outbreaks of *Serratia marcescens* have also been traced to contaminated soap *(2, 5, 27, 30, 37)*. Although no outbreaks in food service have been directly linked to contaminated bulk-soap dispensers, roughly 50% of food service–linked outbreaks can be traced to food workers' hands as the source of pathogens *(16)*. Whereas soaps and other cosmetics are not required to be

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sterile, good manufacturing practices for soaps and cosmetics require that any bacteria present should not constitute a hazard to consumers during regular use *(32)*.

Several factors contribute to bulk-soap contamination, including design of dispenser, soap formulation, and economically motivated dilution of soap *(5, 25)*. To refill sealed dispensers, new cartridges, which contain soap sealed inside with a new nozzle, are placed into the dispenser; in contrast, open refillable bulk-soap dispensers reuse a permanent nozzle and are refilled with soap from a larger bottle. A top-fill reservoir design allows for "topping-off" the soap. Although this potentially reduces soap waste, it also allows mixing of multiple soap lots and types and exposes the soap to an open-air environment, which increases the risk of contamination *(3, 25, 40)*. Furthermore, top-fill design dispensers may never thoroughly be rinsed out, as commonly recommended by dispenser manufacturers. The CDC recommends that bulk liquid soap dispensers be thoroughly cleaned every time before fresh soap is added *(3, 8, 14)*. However, as pointed out by Lorenz et al. *(21)*, no data exist to show that cleanings in between soap refills actually prevent contamination of soap. Regardless, bulk soap can quickly become contaminated due to biofilm formation inside the dispenser (up to 9 log CFU/mL) and can support growth in as little as 24 h *(25)*. Once pump mechanisms are colonized with bacteria, cells from the biofilm continue to contaminate soap, even if completely new bacteria-free soap is used to fill the container *(15)*. Soap formulations will often include preservatives to prevent growth, but because these preservatives are concentration dependent, dilution (as a cost savings measure) can render them ineffective. There has been no evidence of contamination in soap samples collected from dispensers in sealed disposable refills to date.

Potentially harmful bacteria will remain on hands after using contaminated soap *(8, 30, 40)*. Although the bacteria may not be a health concern for the hand washer, these bacteria can transfer from hands to food, objects, and surfaces *(6, 9, 12, 13, 16, 17, 22, 29)*. Hands are one of the main sources of cross-contamination in both health care and food service *(12, 20)*.

The purpose of this study was to survey the microbial quality of open refillable bulk soap sampled in four different food establishment types, within three different states, and to determine the influence of formulation factors on the degree of contamination.

MATERIALS AND METHODS

Sample collection. Samples were acquired from food service establishments around New Brunswick, NJ; Tucson, AZ; and Akron, OH. The categories of merchants from which soap samples were collected were convenience stores, grocery stores, "sit-down" restaurants, and fast food (quick-service) restaurants. Categories were sampled based on the prevalence of the types of establishment in each area and on the likelihood of finding bulk soap in the establishment. Soap was collected from the bathrooms of these establishments. Men's and women's restrooms were sampled in approximately equal frequency. Although soap color was noted, no attempt was made to sample specific colors.

Samples were shipped to the University of Arizona for microbiological analysis, and to GOJO Industries, Inc. (Akron, OH) for physical and chemical analysis. One hundred samples each were collected from Arizona and New Jersey, and 96 samples were collected from Ohio.

Soap samples were collected in a 50-mL sterile conical tube (Corning, Union City, CA), with a minimum volume goal of 45 mL. Two tubes of soap were collected from most establishments, except in a few instances in which a facility only had enough soap for one tube. Soap was collected in the tube by catching the soap released when the dispenser lever was pressed. We used this method to ensure that the soap collected was representative of what would be dispensed onto a customer's hands. Foaming soap was not sampled because bulk refillable foam soap dispensers are uncommon, and challenges in collecting an adequate mass of foaming soap made sampling impractical. Samples were sealed using parafilm (Bemis NA, Neenah, WI) and were placed in an ice pack–chilled cooler after collection.

Microbiological analysis. Total heterotrophic viable bacteria were assayed on Reasoner's 2A agar (R2A; EMD Chemicals, Inc., Gibbstown, NJ), using serial dilutions of 10^{-1} through 10^{-3} of the soap samples, with colonies counted after 5 days of incubation at 22 ± 2°C. R2A agar was originally developed as a rapid method for fecal coliforms in water *(28)*; however, since its development, it has been used in a wide variety of applications, including screening of bulk soap for contaminants *(8)* because it may be especially suitable for culturing slower growing organisms from stressed environments *(36)*. Colonies of the three most predominant morphologies were streaked onto plates of Trypticase soy agar (TSA; EMD Chemicals, Inc.) for isolation and identification. R2A plates were also examined for the presence of *Pseudomonas,* which was then isolated and confirmed.

Coliforms and *E. coli* were quantified using the IDEXX Quanti-Tray/2000 system (IDEXX Laboratories, Westbrook, MA). A 10-mL aliquot of the sample was added to 90 mL of sterile water containing the Quanti-Tray reagent, poured into the Quanti-Tray, and then sealed and incubated at 35°C for 24 h. Coliforms were identified by yellow pigmentation and *E. coli* by fluorescence under UV light. The number of positive yellow and fluorescing wells were quantified, and the IDEXX most-probable-number (MPN) generator program was used for quantification.

Randomly selected coliform-positive wells from the IDEXX Colilert Quanti-Tray/2000 (IDEXX Laboratories) were spread plated on MacConkey agar (EMD Chemicals, Inc.) to select for lactose fermenters. These isolates were then spread plated to TSA (EMD Chemicals, Inc.) and subjected to an oxidase test (BD, Sparks, MD) and API 20E identification biochemical test strips (bioMérieux, Durham, NC) for confirmation as coliforms. Twenty-eight isolates were identified as coliforms and tested for antibiotic resistance by placing antibiotic disks for vancomycin, ampicillin, gentamicin, and ciprofloxacin (Sigma Chemical, St. Louis, MO) onto bacterial lawns of the individual bacteria.

Salmonella preenrichment started by placing a 5-mL aliquot of the soap sample into a tube that contained 10 mL of tryptic soy broth (TSB; EMD), followed by incubation at 35°C for 24 h. After 24 h, 1 mL of the TSB was transferred to a tube that contained 10 mL of Rappaport-Vassiliadis broth (Hardy Diagnostics, Santa Maria, CA), followed by incubation at 41.5°C for 24 h. One milliliter of TSB was also added to a tube that contained 10 mL of selenite cystine broth (EMD Chemicals, Inc.) and was incubated at 35.0°C for 24 h. Each tube showing turbidity was streaked onto plates of Hektoen (EMD Chemicals, Inc.) and xylose lysine desoxycholate (XLD; EMD Chemicals, Inc.) agars and incubated

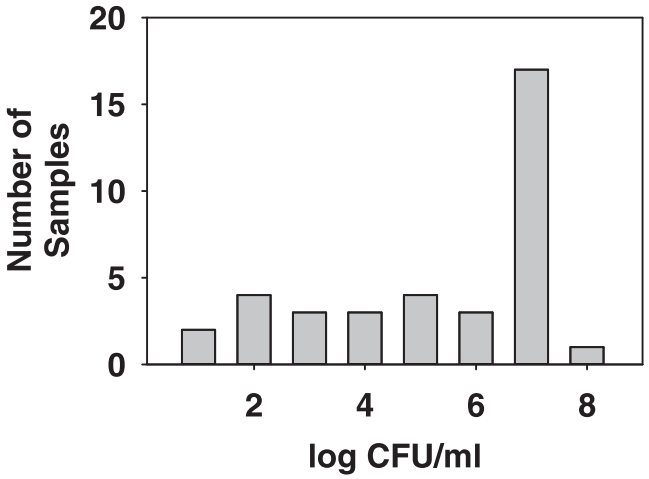

FIGURE 1. *The distribution of microbial counts in contaminated soap samples.*

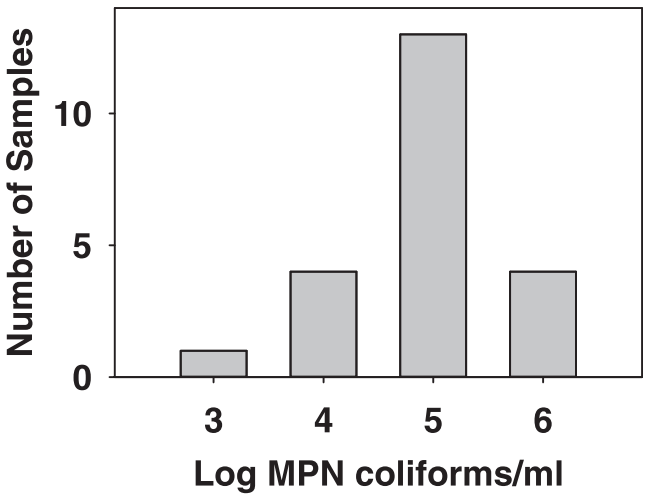

FIGURE 2. *The distribution of coliform counts in contaminated soap samples.*

at 35°C for 24 h. Presumptive *Salmonella* isolates were transferred to TSA for biochemical identification using the API 20E (bioMérieux). If the isolate was presumptively identified as *Salmonella,* the isolated colonies were sent to the National Veterinary Services Laboratories (Ames, IA) for serotyping.

pH and water activity. The pH of all samples was evaluated using a Thermo Orion 720A+ pH with the Thermo Scientific Orion ROSS Sure-Flow pH electrode (Thermo Fisher Scientific, Pittsburgh, PA). Five grams of each test sample was evaluated using the Ohaus standard moisture analyzer (model MB45, Ohaus, Pine Brook, NJ).

A water activity meter (Rotronic Instrument Corp., Hauppauge, NY) was used to measure the water activity of soap samples. Distilled water and glycerol solutions were used as standards. Each sample cup was filled with about 10 mL of soap sample, and after 4 to 5 min the temperature and water activity were recorded. The sample cup was rinsed using distilled water and was dried completely using a Kimwipe (Kimberly-Clark, New York, NY) after each test.

Antimicrobial analysis. All samples were evaluated for the presence and quantity of triclosan using the Waters (Milford, MA) e2695 Alliance high-performance liquid chromatography system with a UV/Visible Detector (Waters 2489) and a Waters μBondapak C18 column (125Å, 10 μm, 3.9 by 150 mm; Waters no. WAT086684). All samples that tested negative for the presence of triclosan were evaluated for the presence and quantity of parachlorometaxylenol, using the same system, detector, and column as used for triclosan.

RESULTS

Most of the soap samples tested (>85%) contained no detectable microorganisms (10 CFU/mL detection limit). The distribution of microbial counts found in contaminated soap samples is shown in Figure 1. Samples containing detectable microorganisms were most often contaminated at a very high level (~7 log CFU/mL), with counts on the remaining samples ranging uniformly from 1 to 6 log CFU/mL. Although not all bacteria recovered were identified, microorganisms detected in contaminated soap included *Klebsiella oxytoca, Serratia liquefaciens, Shigella sonnei, Enterobacter gergoviae, Serratia odorifera,* and *Enterobacter cloacae*. Four of the soap samples were positive for *Salmonella* by API 20E, but were not confirmed as *Salmonella* by the National Veterinary Services Laboratories. Twenty-three samples contained vancomycin-resistant organisms. Seven of these were also resistant to ampicillin, and two of those, in turn, were resistant to gentamicin. One sample contained an organism resistant to vancomycin, ampicillin, gentamicin, and ciprofloxacin (antibiotic resistance data not shown).

When a sample contained detectable coliforms, similarly, the population was likely to be high, as shown in Figure 2. The distribution of coliforms is likely higher than what is shown in Figure 2, because the two highest populations were at the upper limit of quantification (i.e., >241,960 MPN/mL or >24,196 MPN/mL).

Figure 3 shows that higher coliform counts tended to be associated with samples that contained higher bacterial counts overall. Coliform counts at the upper limits of the MPN method are especially associated with high total bacterial counts.

Figure 4 shows the relationship between sample pH and the population of detectable microorganisms. Of samples with a pH less than 7.0, 18% had detectable contamination, whereas only 10% of samples with a pH of 7 and above had detectable contamination. Note, however, that contaminated soap samples with a pH ≥7.0 are more likely to result in contamination at a relatively higher level (i.e., >1,000 CFU/mL), perhaps because pH influences bacterial growth or survival.

Figure 5 shows the relationship between the measured percent solids (top panel) or water activity (bottom panel) of a sample and the bacterial count. Note that every sample containing less than 4% solids had some detectable level of bacteria, whereas only two samples with greater than 14% solids had detectable bacteria. A similar pattern is shown with water activity (Fig. 5, bottom panel), and samples with water activities between 0.99 and 1.0 were associated with a range of bacterial populations, including the highest populations observed. As the measured water activity

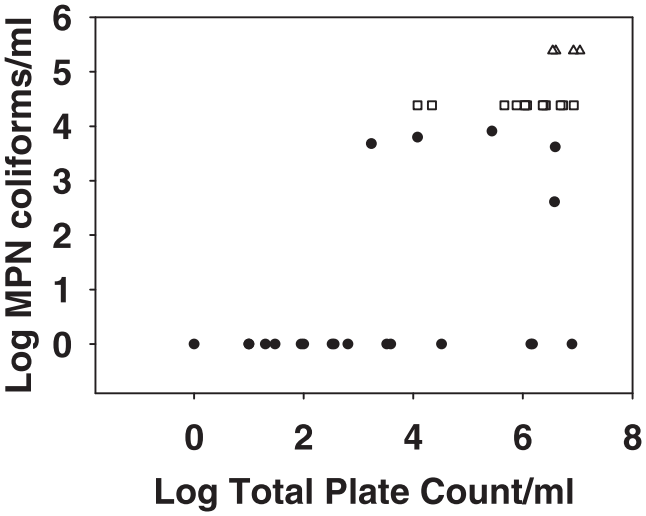

FIGURE 3. *Relationship between coliform counts and total plate counts in contaminated soap samples. Coliform counts above 4.4 log MPN or above 5.4 log MPN are shown using open squares and open triangles, respectively. Counts below the detection limit (10 CFU/mL) are plotted as 0 log CFU or MPN.*

decreased, the occurrence of higher bacterial populations declined, although there was a low population of bacteria in the soap with the lowest water activity measured. There was no clear relationship between the solids content and the water activity (data not shown).

Figure 6 expands upon the analysis of the relationships between percent solids (top panel) or water activity (bottom panel) and bacterial count. As percent solids increases, the fraction of samples with a bacterial count above the detection limit (10 CFU/mL) decreases (Fig. 6, top panel). Note that the two leftmost bars in the figure are associated with very few observations (three and six observations, respectively), whereas all other points are always associated with 30 or more observations. The bottom panel of Figure 6 shows the number of samples associated with different water

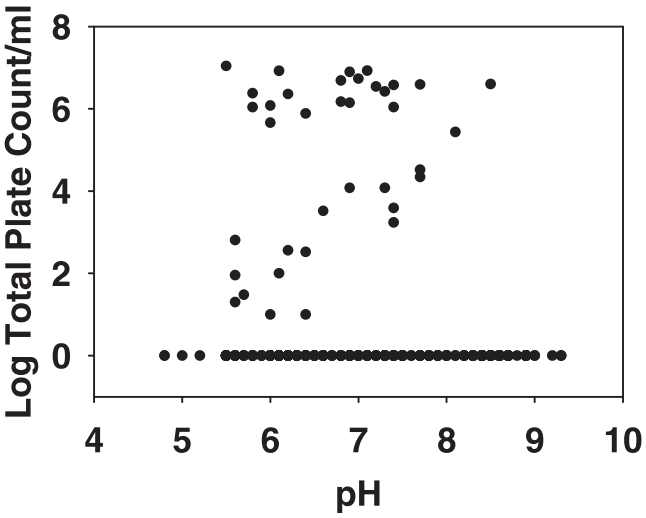

FIGURE 4. *Relationship between sample pH and the population of detectable microorganisms. Counts below the detection limit (10 CFU/mL) are plotted as 0 log CFU.*

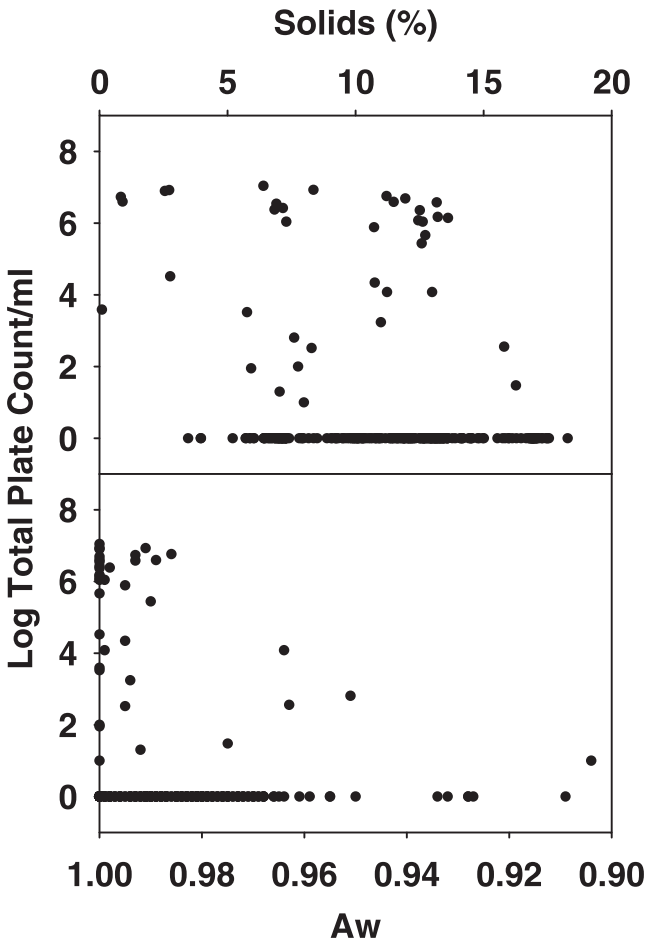

FIGURE 5. *Relationship between soap sample percent solids (top panel) or water activity (bottom panel) and bacterial count. Counts below the detection limit (10 CFU/mL) are plotted as 0 log CFU.*

activities, with the number of samples generally decreasing as water activity increases. The number of contaminated (gray) versus uncontaminated (black) samples are shown by shading on the bars. Clearly the greatest number, as well as the greatest fraction, of samples containing detectable bacteria is associated with higher (0.99 to 1.00) water activities, although even soaps with lower water activity can also contain detectable bacteria.

Figure 7 shows the relationship between the measured population of antimicrobial agent in the soap and the bacterial count. Samples containing no detected antimicrobial agent have widely distributed contamination levels. Although samples containing triclosan were contaminated regardless of the triclosan level (~0.15 to 0.65%), only one sample containing parachlorometaxylenol was contaminated, and that was at a relatively low level (0.15%).

Table 1 shows a summary of these antimicrobial data. Most of the samples tested contained no detected antimicrobial, and these samples contained the greatest fraction with countable microorganisms, almost 17%. There was a statistically significant difference between the fraction of bacteria-positive samples with no detected antimicrobial agent and those containing an antimicrobial agent ($P = 0.0035$). There was not a statistically significant difference between the fractions of bacteria-positive samples for the two types of

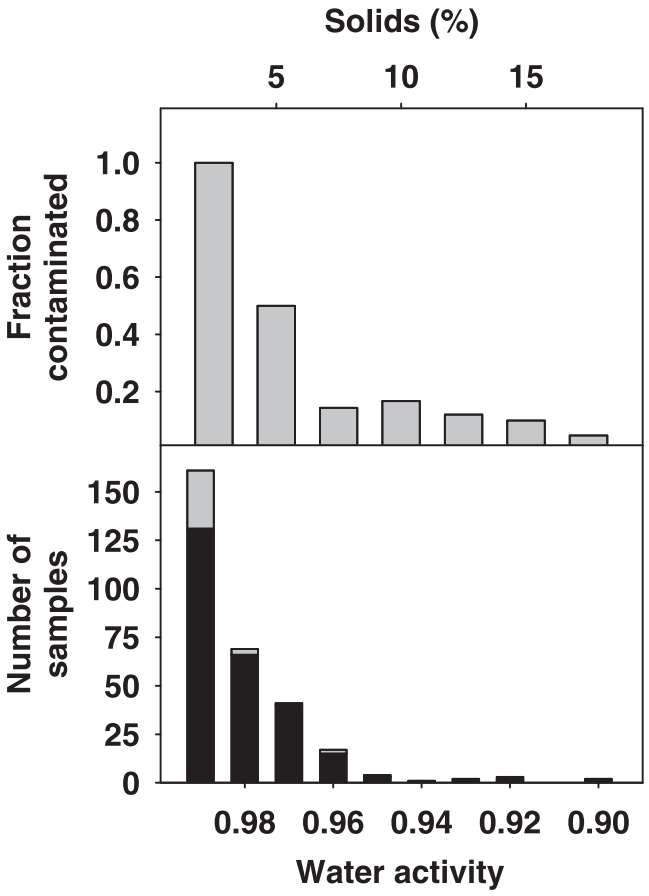

FIGURE 6. *Relationship between fraction of soap samples with bacterial counts above the detection limit (10 CFU/mL) and percent solids (top panel) or number of soap samples contaminated (gray) or uncontaminated (black) and soap water activity (bottom panel).*

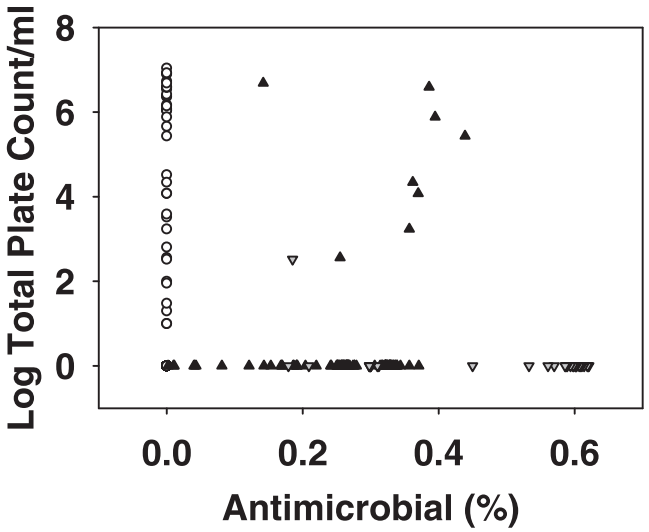

FIGURE 7. *Relationship between the measured concentration of the antimicrobial agent triclosan (black triangle) or parachlorometaxylenol (gray downward triangle) or no detectable antimicrobial agent (open circles) and total bacterial count. Counts below the detection limit (10 CFU/mL) are plotted as 0 log CFU.*

TABLE 1. *Comparison of the fraction of samples containing detectable bacteria for soap samples with detectable antimicrobial agents*[a]

	No. sampled	No. countable	% total samples	% countable
None	166	28	56.1	16.9 A
Triclosan	97	8	32.8	8.2 B
Parachlorometaxylenol	33	1	11.1	3.0 B
Total	296	37	100.0	12.5

[a] Percent countable values followed by a different letter are significantly different ($P < 0.05$).

antimicrobial agents ($P = 0.1022$). The fraction contaminated in total for all soap samples collected was 12.5%.

The relationship between the type of location sampled and the fraction of the time that samples contained detectable microorganisms is shown in Table 2. Grocery stores and fast food operations each had more than 10% bulk-soap samples positive. Grocery stores, fast food restaurants, and sit-down restaurants did not have significantly different fractions of contaminated samples from one another ($P > 0.05$), but grocery stores and fast food restaurants had significantly more ($P < 0.05$) contaminated bulk-soap samples than convenience stores.

The breakdown of bulk-soap samples in Table 3 shows that both men's and women's bathrooms have contaminated soap >10% of the time. Although samples collected from men's restrooms have a slightly higher frequency of detectable bacteria, the difference was not significant ($P = 0.29$).

The relationship between soap color and the presence of detectable bacteria is shown in Table 4. There are differences in the fraction of samples containing detectable bacteria, by soap color. However, given the wide array of soap colors observed, and the small number of samples containing detectable microorganisms, no differences were statistically significant.

Table 5 shows the fraction of samples containing detectable microorganisms by state, with >10% of soap contaminated in all three states. There were not statistically significant differences among the three states where soap samples were collected ($P > 0.05$).

DISCUSSION

This study identified gram-negative organisms as the primary organisms that colonize bulk-soap dispensers,

TABLE 2. *Fraction of samples containing detectable bacteria by store type*[a]

Type	No. sampled	No. of times bacteria detected	% detected
Grocery	30	5	16.7 A
Fast food	122	19	15.6 A
Sit down	113	11	9.7 AB
Convenience	28	1	3.6 B

[a] Percent detected values followed by a different letter are significantly different ($P < 0.05$).

TABLE 3. *Fraction of samples containing detectable bacteria by restroom gender type*

Type	No. sampled	No. of times bacteria detected	% detected
Men	169	23	13.6
Women	114	13	11.4
Other[a]	13	1	7.7

[a] Includes unknown, not recorded, and unisex bathrooms.

consistent with past outbreaks *(1, 2, 24, 38)* and screening studies *(8, 25)*. We identified gram-negative organisms at a broad range of populations (1 to 7 log CFU/mL), as reported by Momeni et al. (2 to 9 log CFU/mL *(25)*). Whereas Momeni et al. found detectable bacteria in ~60% of their samples, we found detectable bacteria in 15% of samples. This may be owing to differences in sample size (our 296 versus their 14), locations (three states versus two institutes), and type of facility (food service versus dental institute). Chattman et al. *(8)* collected 541 bulk-soap samples from five U.S. cities (Boston, Atlanta, Columbus, Los Angeles, Dallas), from liquid soap dispensers in a wide variety of public settings: offices, health clubs, restaurants, and retail stores. These authors found heterotrophic and coliform populations greater than ~2 log CFU/mL in ~19% of the sink area dispensers, similar to what we found (>2 log CFU/mL in ~15% of dispensers).

Specifically relevant to the food industry was the identification of *S. sonnei* from a contaminated soap dispenser in Arizona. According to the CDC, *S. sonnei* is the predominant cause of shigellosis in industrialized countries (and is the most common species in the United States). Consumption of ready-to-eat food contaminated due to handling by an infected worker could be a significant contributor to the spread of *S. sonnei (4)*.

The published literature reports that bacteria are more commonly isolated from plain soaps *(1, 5, 27, 30)* and are less frequently isolated from antimicrobial soaps *(1, 2, 24)*, which is consistent with the findings from our study. Although fewer bacteria are generally isolated from antimicrobial soaps (as they were in our study), it is a major technical challenge to maintain the activity of active ingredients, such as triclosan, so that they are not bound by the surfactant micelles *(11, 34)*. Our research clearly shows that the presence of an antimicrobial agent is not a safeguard

TABLE 4. *Fraction of samples containing detectable bacteria by soap color*

Color	No. sampled	No. of times bacteria detected	% detected
Green	11	5	45.5
Clear	24	7	29.2
Orange	37	8	21.6
Pink	120	12	10.0
White	41	3	7.3
Blue	42	3	7.1
Yellow	16	0	0.0
Unknown	6	0	0.0

TABLE 5. *Fraction of samples containing detectable bacteria by state*

State	No. of samples	No. of times bacteria detected	% detected
AZ	100	14	14.0
NJ	100	11	11.0
OH	96	12	12.5

against the colonization of bulk soap by bacteria. This is consistent with Archibald et al. *(1)*, who detected *S. marcescens* in 1% chloroxylenol soap (parachlorometaxylenol), and with Barry et al. *(2)* and McNaughton et al. *(24)*, who isolated bacteria from soap that contained triclosan.

It is well understood by chemists that formulation affects the performance of hand hygiene products *(10, 23)*. Our study is a reminder that quality also matters in soap development. For example, high water–low solids formulations may be less expensive to manufacture, but they are more likely to be contaminated. Soap delivery systems designed to allow mixing (or dilution of soaps to save money) promote colonization and lead to less-stable formulations. We also observed differences among types of food establishments. Fast food and grocery stores are more likely to be contaminated than convenience stores; this may be because, in the former, there is less maintenance and management oversight of the bathrooms, whereas convenience stores typically have small bathrooms that are cleaned frequently. Fast food restaurants should be of the greatest concern because food handlers often use the bathrooms we sampled that were located in the "front of the house" and then often return directly from the bathroom into the kitchen. This finding warrants strong consideration of Food Code restrictions on the use of bulk soap in restaurants, analogous to rules that discourage their use in health care *(3, 31, 39)*.

We believe this work is generalizable across the United States. Samples were obtained from a variety of food handling environments in three states spread across the country, with a wide range of weather (temperature and humidity); we found no significant differences in level of microbial contamination among states. Our findings show that the design of open refillable systems for dispensing bulk soap is fundamentally flawed and creates opportunities for contamination and biofilm development, independent of geographic location. Future needs and opportunities include better understanding the relationship of bathroom design (e.g., toilet proximity to the soap dispenser, size of bathroom) and further assessment of the risk of antibiotic-resistant bacteria in bulk soaps. Alternative approaches to achieve a lower or acceptable cost to the food service provider are also important, because low cost is the primary attraction to bulk-soap systems. Changing this practice will require good policy development, analogous to what happened in health care *(3)*.

Use of refillable bulk-soap dispensers is a clear public health concern because they provide an environment for bacteria to grow, often to high populations *(8, 21, 25, 30, 40)*, and their use has led to non–foodborne disease outbreaks *(1, 2, 5, 24, 27, 30, 38)*. In our study, most soap

samples had no detectable bacteria; however, those soap samples that did have detectable bacteria (12.5%) had populations that would be considered highly risky if the bacteria present were pathogenic (~7 log CFU/mL). Whereas the CDC recommends that bulk liquid soap dispensers be thoroughly cleaned before adding fresh soap *(3, 8, 14),* cleanings in between soap refills might not prevent recontamination *(21),* and difficult-to-clean biofilms may develop. Bulk soap has been proven to cause infection outbreaks in health care settings. It has been difficult to document outbreaks in food service settings to date; however, our findings show that the use of bulk soap presents a clear risk in food service facilities.

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