Occurrence of Heterotrophic and Coliform Bacteria in Liquid Hand Soaps From Bulk Refillable Dispensers in Public Facilities

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Abstract **The goal of the study discussed in this article was to determine the occurrence of heterotrophic and coliform bacteria in liquid soap from bulk refillable dispensers, obtained from restrooms in a variety of public facilities. A total of 541 samples was collected from five U.S. cities. Liquid soap from dispensers in public areas was found to contain heterotrophic and coliform bacterial numbers averaging more than 106 CFU/mL in 24.8% of the dispensers.**

Introduction

Liquid hand soap is used daily by millions of people worldwide. Some public restrooms have soap dispensers that require bagged or cartridge-sealed soap replacements and others have containers that are refillable using stock soap solutions that are often diluted with tap water. Although liquid hand soaps are not required to be sterile, the microbial burden is to be kept to a minimum and free of objectionable microorganisms. They must also be adequately preserved to prevent contamination once in the hands of the consumer.

A study conducted in Japan in the early 1990s by Amemiya and Taguchi (1992) revealed significant contamination in soap from public restrooms. They found that as many as 4 x 10^7 bacteria per mL could be recovered from liquid hand soaps and that 71% contained 1,000 or more bacteria per mL. Until now, no large survey studies of this kind have been done in the U.S. The purpose of our study was to assess the frequency and extent of bacterial contamination in soaps from open refillable dispensers commonly used by patrons of public restrooms across the U.S. We also identified organisms that were found in order to determine whether the use of soap from public restrooms poses a potential health hazard.

Materials and Methods

Liquid soap samples were collected from public restrooms in five cities (Boston; Atlanta; Columbus, Ohio; Los Angeles; and Dallas). Sample locations were organized into four categories: offices, health clubs, restaurants, and retail stores. Soap was collected from refillable soap dispensers (*N* = 541) into sterile 50 mL centrifuge tubes through the soap dispenser mechanism. The soap samples were shipped on ice to the University of Arizona and processed the same day they were received. Approximately 20% of the individual sampling sites were independently audited within 30 days after initial collection. The auditors were not the same persons who collected the initial soap samples. The auditors verified the accuracy of the information recorded by the original collector and that all of the samples had been obtained from bulk through-the-counter or wall-mounted refillable dispensers.

One mL of Dey-Enger (DE) neutralizing broth was added to each sample tube and shaken for 30 seconds. Heterotrophic plate counts (HPC) were obtained by spread plating 0.1 mL of sample onto duplicate petri dishes containing R2A media. Plates were incubated at 30°C for five days. After incubation, the colonies were counted and the mean values were recorded for each set of duplicates. Plates containing colonies that were too numerous to count (>300 CFU/plate) were reassayed the next day by performing 10-fold dilutions of the soap in sterile buffered saline (0.85% NaCl). The dilutions were then plated onto duplicate petri dishes containing R2A agar and incubated at 30°C for five days. Plates were counted and the HPC for each soap sample was calculated. Any soap sample that contained more than 500 CFU/mL was also assayed for the presence of coliform bacteria and *Staphylococcus aureus* bacteria. The cutoff of 500 CFU/mL was chosen because it represents the maximum microbial burden in cosmetic products as recommended by the Personal Care Products Council (PCPC).

Coliform enumeration was performed by spread plating the appropriate dilution of each of the contaminated soap samples on mEndo agar plates and incubated at 37°C for 24 hours. Representatives of each colony type were streaked for isolation on petri dishes containing tryptic soy agar (TSA) in order to perform oxidase tests and identification. TSA plates were incubated at 35°C for 24 hours. Oxidase tests were performed on all isolates by applying Kovacs reagent. A positive control (*Pseudomonas aeruginosa*, American Type Culture Collection [ATCC] #27313) and a negative control (*E. coli*, ATCC #25597) ensured the quality of the Kovacs reagent.

TABLE **1**

Occurrence of Bacteria in Liquid Hand Soap From Refillable Dispensers

Location	# of Liquid Soap Samples	# > 500 CFU/mL	# Containing Coliform Bacteria	% of Samples With HPC > 500 CFU/mL	% of Samples Containing Coliform Bacteria
Total	541	134	86	24.8	15.9
Sink area	428	83	56	19.4	13.1
Shower area	113	51	30	45.1	26.5

TABLE **2**

Frequency of Detection of Bacteria in Liquid Hand Soaps

Bacterial Species	Frequency of Detection
Klebsiella oxytoca	30
Klebsiella pneumoniae	29
Enterobacter aerogenes	13
Serratia marcescens	12
Pseudomonas aeruginosa	7
Citrobacter koseri / farmeri	3
Enterobacter cloacae	2
Enterobacter gergoviae	2
Serratia odorifera	1
Serratia liquefaciens	1
Pantoea spp.	1
Klebsiella ornithinolytica	1
Enterobacter spp.	1
Citrobacter freundii	1
Enterobacter sakazakii	1

S. aureus analysis was performed by spread plating the appropriate dilution of the original sample on TSA amended with 5% sheep blood (blood agar) to check for hemolysis. Plates were incubated at 35°C and examined after 24 and 48 hours. Beta hemolytic isolates were enumerated and streaked on petri dishes containing TSA and incubated for 24 hours at 35°C. Confirmation testing of isolated colonies included catalase production tests, coagulase production tests (tube and slide), antibiotic (polymyxin) sensitivity, and analysis of microscopic morphology.

Identification of bacteria was performed by using API20E strips. Five mL of sterile saline (0.85% NaCl) were aseptically transferred to a sterile 10 mL capped tube. Using a sterile cotton swab, several isolated colonies from a 24-hour-old culture were resuspended in the saline solution. The API20E strip was then inoculated with the bacterial suspension. The strip was incubated at 35°C for 24 hours. The necessary reagents were added and the API20E strip was interpreted.

Results

The total number of liquid soap samples analyzed was 541, consisting of 428 from sink areas and 113 from showers (Table 1). The percentage of samples that contained HPC numbers above 500 CFU/mL was 24.8%, averaging 3.0 x 10^6 CFU/mL and ranging from 590 to 1.3 x 10^7 CFU/mL. Total coliform bacteria were detected in 15.9% of the samples, averaging 3.9 x 10^6 CFU/mL and ranging from <10 CFU/mL to 6.5 x 10^7 CFU/mL. Table 2 itemizes the different species of Gram-negative bacteria that were isolated. Species of *Klebsiella* occurred most frequently, followed by *Enterobacter*, *Serratia*, and *Pseudomonas*. No *S. aureus* was detected in any of the liquid soap samples analyzed. Office restrooms had the highest percentage of contamination with heterotrophic bacteria (47.5%) and coliform bacteria (35.0%) and restrooms in retail stores had the least (15.3% for heterotrophic and 10.6% for coliform bacteria) (Table 3). The rates of contamination of soap were similar among all five metropolitan areas (Table 4).

Discussion

The results of our study indicate that liquid soap in public restrooms from bulk refillable dispensers is frequently contaminated with bacteria at levels much higher than maximum recommend levels. All of the organisms detected in the soap samples were Gram-negative bacteria. This is most likely because of the presence of sodium lauryl sulfate (SLS) in the soap, which inhibits Gram-positive bacterial growth. In fact, SLS is used to inhibit Gram-positive growth in selective media such as mEndo agar (Difco Laboratories, 1998). All of the organisms that were identified in our study were Gram-negative opportunistic pathogens. The opportunistic pathogens most commonly found in liquid hand soap included *Pseudomonas* spp., *Serratia marcescens*, and *Klebsiella pneumoniae*. These bacteria are also among the most prevalent organisms that cause opportunistic infections. These were also the same species of bacteria that Amemiya and Taguchi (1992) isolated most often in liquid soap in Japan. While largely associated with infections in compromised patients (immunocomprimised, burn patients, post-surgical) they are capable of causing infections of wounds (cuts to the skin), folliculitis, and urinary tract infections (Dissemond, Schmid, Esser, Witthoff, & Goos, 2004; Kallman, Lunderberg, Wretling, & Ortqvist, 2006; Swartz, 2000).

TABLE **3**

Occurrence of Bacteria in Liquid Hand Soaps Collected From Different Locations

Location	# of Liquid Soap Samples	# > 500 CFU/mL	# Containing Coliform Bacteria	% of Samples With HPC > 500 CFU/mL	% of Samples Containing Coliform Bacteria
Restaurants	98	17	15	17.3	15.3
Retail	216	33	23	15.3	10.6
Gyms/health clubs	187	65	34	34.8	18.2
Offices	40	19	14	47.5	35.0

TABLE **4**

Occurrence of Bacteria in Liquid Hand Soap by City

Location	# of Liquid Soap Samples	# > 500 CFU/mL	# Containing Coliform Bacteria	% of Samples With HPC > 500 CFU/mL	% of Samples Containing Coliform Bacteria
Boston	107	27	18	25.2	16.8
Los Angeles	94	24	14	25.5	14.9
Dallas	111	30	28	27.0	25.2
Columbus, OH	109	26	19	23.9	17.4
Atlanta	120	27	7	22.5	5.8

It has also been reported that bacteria present in the liquid soap remain on the hands after use. Sartor and co-authors (2000) found that after hand washing with an *S. marcesens*–contaminated liquid hand soap pump, the hands of health care workers were 54 times more likely to be contaminated with *S. marcescens*. Infections and outbreaks resulting from the use of contaminated soap or disinfection products are not uncommon in health care settings. Several different types of soaps contaminated with *S. marcescens* have caused a variety of infections including bacteremia, conjunctivitis, meningitis, and joint infections. Species of *Pseudomonas* and *Burkholderia* found in soap have been linked to various outbreaks in hospitals and infections including skin ulcers, bacteremia, and urinary tract infections (Archibald et al., 1997; Dolan, Eberhart, & James, 2006; McNaughton, Mazinke, & Thomas, 1995; Sartor et al., 2000). Hand lotion contaminated with *P. aeruginosa* was implicated as the vector resulting in hand transfer of the organism to infected infants (Becks & Lorenzoni, 1995).

Infections and outbreaks in hospitals linked to the use of contaminated products raised awareness of the associated health hazards that subsequently led to improvements in products and hygiene practices. Throughout the 1960s to 1980s, for example, numerous studies consistently reported that bar soap is frequently contaminated with pathogenic bacteria, some of which were implicated in infections (Kabara & Brady, 1984). Liquid soap was shown to be the safer alternative. Dispenser systems were developed in part to respond to this identified need for more rigorous hygiene requirements (Graf, Kersch, & Scherzer, 1988). In the hospital setting, however, it was found that product contamination could occur in liquid soap dispensers. Graf and co-authors (1988) found that rapid recontamination of fresh product resulted from insertion of a soap pump top into new base bottles of soap. The Centers for Disease Control and Prevention (CDC) recognized this issue and recommended in 1975 that liquid soap dispensers be thoroughly cleaned when empty before adding fresh product (Garner & Favero, 1986). Grohskopf and co-authors (2001) found that staff members of a hospital were not emptying and cleaning soap dispensers before refilling, which resulted in an outbreak of *Serratia liquefaciens* in a hemodialysis center. No more infections occurred after it was recommended to use disposable soap containers rather than refillable dispensers.

In another study in which previously healthy individuals suffered severe skin ulcers as the result of infections they received from their soap, the likely culprit was deemed to be a gallon jug hand wash of detergent in which the same nozzle was reused on each new gallon of soap. In addition to lack of adequate cleaning of surfaces that come into contact with the product, other factors that have been shown to contribute to cases of extrinsic soap contamination include the use of contaminated tap water to dilute product, mixing of incompatible product types, and not following suggested product dilution procedures (Amemiya & Taguchi, 1992; McBride, 1984).

Conclusion

At this point it is still uncertain as to exactly how some liquid hand soaps become a suitable environment for bacteria. Most if not all commercially available soaps contain some type of preservative to inhibit microbial growth. It would thus appear that degradation of the preservatives over time is the likely explanation for the occurrence of the bacteria. Our study points out that a need exists for continued research to study bacterial contamination of hand soaps in public rest-

rooms. Future research will be aimed at assessing the public health risk associated with this problem, to determine the factors that result in contamination, and to determine the best methods to reduce the problem. Possible solutions might include better maintenance of the dispensers (cleaning, replacement of soap), use of preservatives more resistant to degradation, or the use of disposable sealed soap refills.

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References

Amemiya, K., & Taguchi, F. (1992). Survey of bacterial contamination of hand washing liquids. *Journal of Antibacterial and Antifungal Agents, 20,* 459–463.

Archibald, L.K. Corl, A., Shah, B., Schulte, M., Arduino, M.J., Aguero, S., Fisher, D.J., Stechenberg, B.W., Banerjee, S.N., & Jarvis, W.R. (1997). *Serratia marcescens* outbreak associated with extrinsic contamination of 1% chlorxylenol soap. *Infection Control and Hospital Epidemiology, 18*(10), 704–709.

Becks, V.E., & Lorenzoni, N.M. (1995). *Pseudomonas aeruginosa* outbreak in a neonatal intensive care unit: A possible link to contaminated hand lotion. *American Journal of Infection Control, 23*(6), 396–398.

Difco Laboratories. (1998). *Difco manual*. Detroit: Author.

Dissemond, J., Schmid, E.N., Esser, S., Witthoff, M., & Goos, M. (2004). Bacterial colonization of chronic wounds—Studies on outpatients in a university dermatology clinic with special consideration of ORSA. *Der Hautarzt 55*(3), 280–288.

Dolan, S.A., Eberhart, T., & James, J.F. (2006). Bubbles to wubbles: An investigation involving the contamination of soap bubble products at a pediatric hospital. *Journal of Specialists in Pediatric Nursing, 11*(3), 189–195.

Garner, J.S., & Favero, M.S. (1986). CDC guidelines for the prevention and control of nosocomial infections—Guideline for hand washing and hospital environmental control, 1985 (Supersedes guideline for hospital environmental control published in 1981). *American Journal of Infection Control, 14*(3), 110–129.

Graf, W., Kersch, D., & Scherzer, G. (1988). Microbial contamination of liquid-soap wall dispensers with one-way bottles. *Zentralblatt fuer Bakteriologie, Mikrobiologie und Hygiene, 186*(2), 166–179.

Grohskopf, L.A., Roth, V.R., Feikin, D.R., Arduino, M.J., Carson, L.A., Tokars, J.I., Holt, S.C., Jensen, B.J., Hoffman, R.E., & Jarvis, W.R. (2001). *Serratia liquefaciens* bloodstream infections from contamination of epoetin alfa at a hemodialysis center. *New England Journal of Medicine, 344*, 1491–1497.

Kabara, J.J., & Brady, M.B. (1984). Contamination of bar soaps under "in-use" conditions. *Journal of Environmental Pathology Toxicology Oncology, 5*(4–5), 1–14.

Kallman, O., Lunderberg, C., Wretlind, B., & Ortqvist, A. (2006). Gram-negative bacteria from patients seeking medical advice in Stockholm after the tsunami catastrophe. *Scandinavian Journal of Infectious Disease, 38*(6–7), 448–450.

McBride, M.E. (1984). Microbial flora of in-use soap products. *Applied and Environmental Microbiology, 48*(2), 338–341.

McNaughton, M., Mazinke, N., & Thomas, E. (1995). Newborn conjunctivitis associated with triclosan 0.5% antiseptic intrinsically contaminated with *Serratia marcescens*. *Canadian Journal of Infection Control, 10*(1), 7–8.

Sartor, C., Jacomo, V., Duvivier, C., Tissot-Dupont, H., Sambuc, R., & Drancourt, M. (2000). Nosocomial *Serratia marcescens* infections associated with extrinsic contamination of liquid nonmedicated soap. *Infection Control and Hospital Epidemiology, 21*(3), 196–199.

Swartz, M.N. (2000). Cellulitis and subcutaneous tissue infections. In G.L. Mandell, J.E. Bennet, & R. Dolin (Eds.), *Principles and practice of infectious disease* (pp. 1037–1057). Philadelphia: Churchill Livingstone.

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