

How Safe is Your Soap?

**Bacterial Contamination
of Soap from Open
Refillable Bulk Dispensers**

Charles P. Gerba, PhD

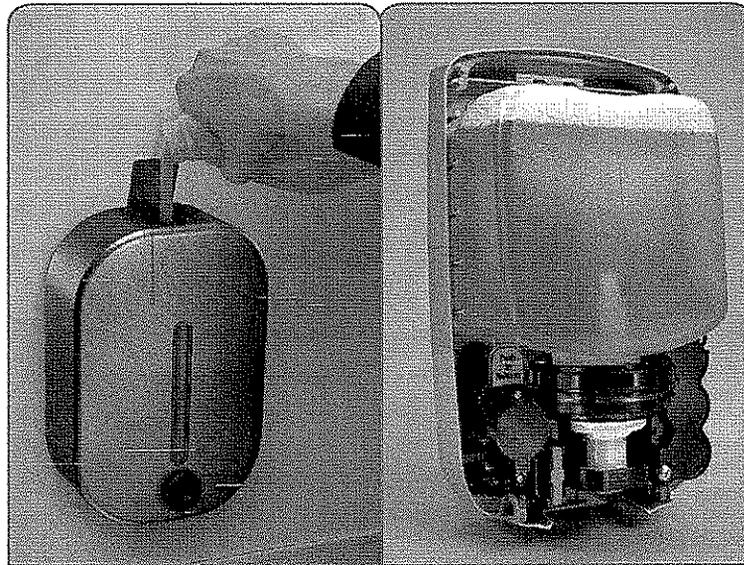
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An overview and summary of research studies conducted by
The University of Arizona, Tucson, AZ, and presented to:

- The American Society for Microbiology 107th General Meeting
Toronto, ON, Canada; May 21-25, 2007
- The National Environmental Health Association 71st Annual
Educational Conference & Exhibition
Atlantic City, NJ; June 18-21, 2007

Do you know the difference?



**Open Refillable
Bulk Soap Dispenser**

**Sealed Soap
Dispensing System**

| | | |
|-------------------------|---|---|
| Design | <ul style="list-style-type: none"> • Open to the environment • Permanent nozzle is reused | <ul style="list-style-type: none"> • Factory sealed • New nozzle with each refill |
| Refilling Method | <ul style="list-style-type: none"> • Pour soap into dispenser from bottle | <ul style="list-style-type: none"> • Snap new cartridge into dispenser |
| Maintenance | <ul style="list-style-type: none"> • Labor intensive • Extensive cleaning and sanitizing required | <ul style="list-style-type: none"> • Labor-free • No need for cleaning and sanitizing |
| Contamination | <ul style="list-style-type: none"> • Prone to contamination | <ul style="list-style-type: none"> • Safe from contamination |

Bacterial Contamination of Soap from Open Refillable Bulk Dispensers

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Bulk Soap Contamination Research Study Summary

Background

Several studies conducted during the last 25 years have demonstrated that liquid soaps can become contaminated with microorganisms and multiple instances of infections and nosocomial outbreaks associated with such contamination have been reported (1-4). Contamination often occurs after the product reaches the user (extrinsic contamination) (1;3;5) and has been observed in both nonmedicated (1) and antimicrobial products including those with the active ingredients Chloroxylenol (PCMX) (3), Benzalkonium chloride (5;6), Triclosan (4), and Chlorhexidine gluconate (2;5;7-10). All types of liquid soap, regardless of the active ingredient or preservative system, are susceptible to contamination when exposed to adverse circumstances. Soap dispensers with sealed disposable refills are an alternative to this contamination challenge. By contrast, open refillable ("bulk") soap dispensers continue to present significant risk of contamination during use. Because the addition of soap

to a partially empty dispenser ("topping off") can lead to bacterial contamination in healthcare settings, the CDC recommends the use of soap dispensed from disposable containers or containers that are thoroughly washed and dried prior to refilling (11;12).

Recent studies conducted at the University of Arizona by prominent microbiologist, Dr. Charles P. Gerba, revealed that liquid hand soap collected from open refillable dispensers are a public health risk. Dr. Gerba determined the levels of bacteria in soap sampled from various types of dispensers. He found unsafe levels of bacterial contamination in soap from open refillable dispensers, whereas no bacterial contamination was found in soap from dispensers with sealed disposable refills. This research has been presented at two recent scientific conferences (13;14).

National Environmental Health Association 71st Annual Educational Conference & Exhibition

Atlantic City, NJ; June 18-21, 2007

Title: Bacterial Contamination of Liquid Hand Soaps Used in Public Restrooms

Authors: C. P. Gerba and S. Maxwell; University of Arizona, Tucson, AZ

Abstract

The objective of this study was to determine the occurrence of heterotrophic and coliform bacteria in liquid hand soaps collected from public restrooms across the United States. Sample locations included public restrooms in restaurants, health clubs, office buildings and retail stores. The liquid soap samples collected were from refillable dispensers (also referred to as "open systems" or "bulk soap" systems). Of 541 samples, 133 (25%) had bacterial numbers greater than 500 CFU/mL and 87 samples (16%) contained coliform bacteria. Approximately 65% of the bacteria isolated from the soap belonged to the coliform group.

The average number of bacteria detected in the soap was 3.02×10^6 CFU/mL with a range of 590 to 5.3×10^7 CFU/mL. The average number of coliform bacteria was 3.94×10^6 CFU/mL with a range of <10 to 6.5×10^7 CFU/mL. Opportunistic pathogens identified in the liquid soap samples included *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Enterobacter sakazakii*. No bacteria were detected in dispensers that required sealed soap replacements. 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 in the soap, which inhibits the growth of Gram-positive bacteria. The results suggest that some liquid soap dispensers become colonized by Gram-negative bacteria over time, possibly because of the degradation of preservatives in the liquid soap.

American Society for Microbiology 107th General Meeting

Toronto, ON, Canada; May 21-25, 2007

Title: Bacterial Contamination of Liquid Hand Soaps

Authors: M. Chattman, S. Maxwell and C. P. Gerba; University of Arizona, Tucson, AZ

Abstract

The occurrence of heterotrophic bacteria (HPC) and coliform bacteria in liquid hand soap from 130 refillable unsealed (a.k.a. open or bulk) dispensers collected from employee break rooms, airplane restrooms, kitchens and public restrooms was determined. The percentage of samples that contained HPC numbers above 500 CFU/mL was 23%, averaging 4.5×10^6 CFU/mL. Total coliform bacteria were detected in 22% of the samples, averaging 2.2×10^6 CFU/mL. Bacterial species most frequently identified included *Serratia marcescens*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae*. One of the soap dispensers containing contaminated soap was monitored over a three month period. Various levels and types of bacterial contamination were observed. When bacteria were added to uncontaminated, factory-sealed, liquid hand soap the bacteria quickly died. Liquid hand soap from a public restroom, that contained large numbers of bacteria was pasteurized

and inoculated with *K. pneumoniae*. Growth was observed, indicating that degradation of preservatives must occur in the soap dispenser over time, allowing for the growth of bacteria. These results demonstrate that bacteria growing in soap dispensers are not resistant to the preservatives and that preservative degradation takes place, likely after introduction of the soap in the dispensers.

Contaminated Bulk Soap is a Public Health Risk

Dr. Gerba's studies demonstrate that soap from open refillable dispensers in public restrooms in the US are routinely contaminated with opportunistic pathogens. Soap users are exposed to an average of over 1,000,000 of these bacteria approximately 1 in 4 times they wash with soap from an open refillable soap dispenser. This level of contamination is 1000 times greater than upper limit recommended by cosmetic industry standards (15) and presents a potential health risk to the soap users as well as to others they may have contact with. Hands are known to be a common transmission vector and it has been shown that bacteria remain on the hands after using contaminated soap (1). The risk of acquiring an infection is greatest for anyone who has a defect in their body's normal defense mechanisms. Up to 20% of the US general public have impaired immune function and this percentage is growing due to advances in medicine which are prolonging life as well as the increase in the proportion of elderly in the population (16-18). The immunocompromised population includes a diverse group with a wide variety of conditions ranging from the severely immunocompromised (HIV/AIDS, cancer, organ or bone marrow transplant recipients) to pregnant women, young children and the elderly which exhibit non-specific general reduced immune function (19;20). The fetus, neonate

and young children have reduced immune function for the first few years of life until their immune systems mature (19). Over 12% of the US population is over the age of 65 and are at a greater risk of acquiring infections due to their age-related diminished immunity (16;21). In addition, many common chronic conditions weaken the immune system including diabetes (18), cirrhosis/alcoholism, chemical dependency, nutritional deficiencies, and any defects resulting in skin barrier function loss (burns, ulcers, or dermatitis) (17;20).

Illnesses Caused by the Contaminating Bacteria

In the recent study by Dr. Gerba, several of the bacterial species isolated from the contaminated soap (e.g. *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, and *Pseudomonas*) are medically important opportunistic pathogens. These organisms cause a variety of illnesses including respiratory tract infections, pneumonia, urinary tract infections, bloodstream infections, surgical site infections, meningitis, skin ulcers, gastroenteritis as well as wound and soft tissue infections (22-24). *Klebsiella pneumoniae*, for example, is responsible for 1-5% of community-acquired pneumonia (25). *Enterobacter sakazakii* causes neonatal meningitis (26). *Citrobacter* causes sepsis, meningitis and central nervous system abscesses in neonates and young infants (27) and there has been one report of *Citrobacter koseri* causing a central nervous system infection in a healthy person with a fully functional immune system (28). *Citrobacter freundii* was also implicated as a potential cause of an outbreak of diarrheal disease (24). *Pseudomonas aeruginosa* is a common nosocomial pathogen causing urinary tract infections, sinusitis, wound infections, and pneumonia. Occasionally it has been known to cause a rare form

of community-acquired pneumonia with a 33% mortality rate that can affect persons with healthy immune systems (29). *Serratia* has been implicated in multiple outbreaks due to contaminated soaps in healthcare facilities (1;3;4). *Pañtoea* is a rare pathogen that was reported to be responsible for 7 infant deaths in a neonatal outbreak (30). The frequent presence of such high numbers of organisms known to be medically significant both in the community and in healthcare settings is quite alarming.

Reducing the Risks of Bulk Soap Contamination

Unsafe levels of contamination were found in 23% - 25% of soap samples collected from open refillable dispensers. In contrast, no contamination was found in soap samples collected from dispensers containing sealed disposable refills. It is recommended that all open refillable dispensers should be switched to dispensers with sealed disposable refills, which are a safer alternative and avoid unnecessary health risk.

Bacterial Contamination of Liquid Hand Soaps

Introduction

Liquid hand soap is used daily by millions of people worldwide. Hand washing, with soap and water, is a universally accepted method to reduce the microbial load on the hands. People encounter situations in which they are exposed to a variety of bacteria that have the ability to cause infection. In response to these situations, many people wash their hands with soap and water. Society recognizes that good hygiene can reduce the risk of bacterial infection. Some public facilities have soap dispensers that require sealed bags or cartridges while others have dispensers that are refillable by using stock soap solutions that are often diluted with tap water. Bulk open refillable liquid soap dispensers in many public restrooms and restaurants, offer a suitable environment for the growth of potentially disease causing microorganisms.

Materials and Methods

Soap was collected into sterile 50mL centrifuge tubes through the dispenser mechanism. One mL of Dey-Enger (DE) neutralizing broth (Remel, Lenexa, KS) was added to each sample tube and shaken for 30 seconds. Heterotrophic plate counts (HPC) were obtained by spread plating 0.1mL onto duplicate petri dishes containing R2A media (Difco, Sparks, MD) and incubated at 30°C for 5 days.

Coliform enumeration was performed by spread plating on mEndo agar plates (Difco, Sparks, MD) and incubating at 37°C for 24 hours. Representatives of each colony type were streaked for isolation on petri dishes containing Tryptic Soy Agar (TSA) (Difco, Sparks, MD). Identification of bacteria was performed by using API20E strips (BioMerieux, Marcy-l'Etoile, France).

Results

Table 1: Occurrence of Bacteria in Liquid Hand Soap from Refillable Dispensers

| Type of soap dispenser | Total number of liquid soap samples tested | Number >500 CFU/mL | Coliform bacteria | Average number HPC CFU/mL | Average number coliform bacteria CFU/mL |
|------------------------|--|--------------------|-------------------|---------------------------|---|
| Refillable | 132 | 30 | 29 | 4.5×10^6 | 2.2×10^6 |
| Disposable bag | 20 | 0 | 0 | 0 | 0 |

Figure 1: Frequency of Detection of Various Bacteria in Soap Samples

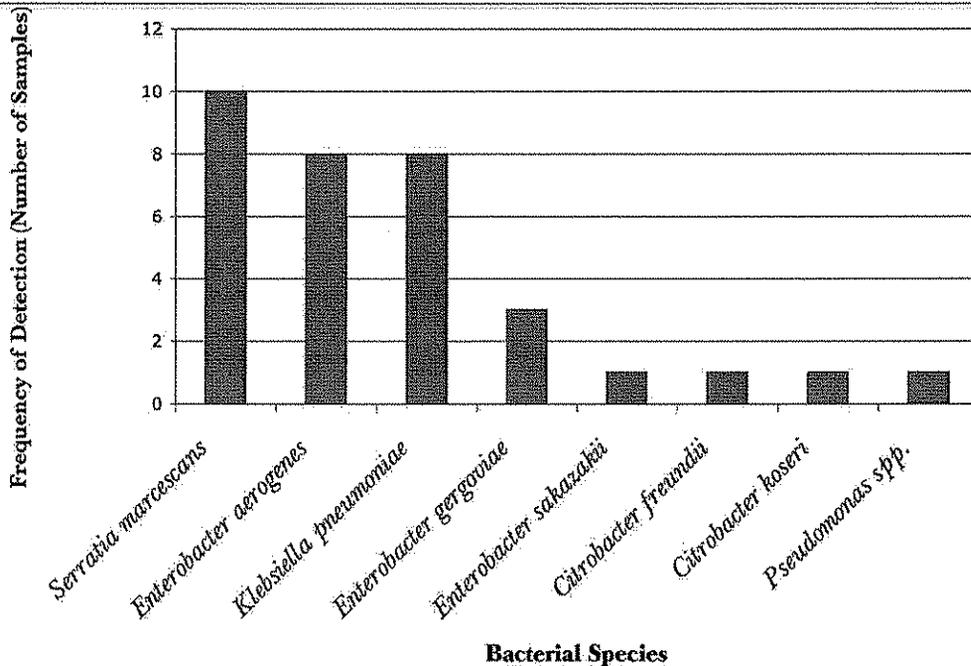


Table 2: Occurrence of HPC and Coliform Bacteria Over Time in a Restaurant Soap Dispenser

| Date | HPC (CFU/mL) | Coliform bacteria (CFU/mL) |
|------|-------------------|----------------------------|
| 5/29 | 4.3×10^7 | 2.0×10^7 |
| 6/21 | 9.3×10^3 | <1 |
| 7/17 | 1.9×10^7 | 8.2×10^6 |
| 7/31 | 9.5×10^6 | 7.3×10^6 |
| 8/14 | 1.2×10^7 | 9.0×10^6 |
| 8/28 | <1 | <1 |
| 9/12 | 3.2×10^7 | 2.6×10^7 |

Table 3: Growth of *Klebsiella pneumoniae* in Pasteurized Contaminated Soap (CFU/mL)

| Sample | Time (Days) | | | |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| | 0 | 1 | 4 | 8 |
| 9/12 | 6.3×10^2 | 3.4×10^4 | 5.9×10^5 | 2.5×10^6 |
| Negative Control* | <1 | <1 | <1 | <1 |

* Undiluted, pasteurized soap inoculated with same amount of bacteria as sample. Number of *Klebsiella pneumoniae* added to each soap sample was 5.6×10^3 (9.3×10^2 CFU/mL).

Table 4: Minimum Inhibitory Concentration of a Liquid Soap against *Klebsiella pneumoniae* (CFU/mL)

| Dilution | 0 | Time (Days) | | | |
|-------------------|-----|-------------|-----|-------------------|-------------------|
| | | 60 min | 1 | 5 | 10 |
| No Dilution | 50 | <1 | <1 | <1 | <1 |
| 1:1 | 330 | <1 | <1 | <1 | <1 |
| 1:2 | 490 | <1 | <1 | <1 | <1 |
| 1:4 | 480 | 160 | <1 | <1 | <1 |
| 1:10 | 410 | 320 | <1 | <1 | <1 |
| 1:100 | 350 | 580 | 240 | <1 | <1 |
| 1:1000 | 410 | 550 | 240 | 1.5×10^4 | 1.5×10^5 |
| Negative Control* | 500 | 520 | 150 | 1.5×10^3 | 2.3×10^3 |

* No soap added.

Conclusions

- 22.7% of samples taken from refillable bulk dispensers contained >500 CFU/mL HPC, and 22% contained coliform bacteria, averaging 10^6 CFU/mL.
- Bacterial species identified were all opportunistic pathogens.
- No bacteria were found in sealed system soap dispensers.
- A soap dispenser monitored over a three-month period, demonstrated that bacterial contamination was prolonged although the levels and types of bacteria varied.
- Eight types of uncontaminated, factory-sealed, liquid hand soaps were inoculated with various species of bacteria. All of the bacteria quickly died in the soaps after addition, even when the soap was diluted.
- The minimum inhibitory concentration of a specific brand of soap used at a restaurant that had bacterial contamination in the soap indicated that it contained sufficient concentrations of preservatives to inhibit bacterial growth.
- Liquid soap from a public restroom, that contained large numbers of bacteria was pasteurized and inoculated with *Klebsiella pneumoniae*. Growth was observed, thus it appears that degradation of preservatives must occur in the soap dispenser, allowing for the growth of bacteria.
- Bacteria growing in the soap dispensers are not resistant to the preservatives and that preservative degradation takes place, likely after introduction of the soap into the dispensers.

Bacterial Contamination of Liquid Hand Soaps Used in Public Restrooms

Introduction

Washing hands with soap and water is a universally accepted method to reduce the microbial load on the hands and is used daily by millions of people worldwide. However, the majority of public facilities have soap dispensers that are refillable using a stock soap solution. The CDC recognized in 1975 that the use of these types of dispensers can result in a suitable environment for the growth of potentially disease causing microorganisms. Current health-care hand hygiene guidelines do not recommend the use of open refillable dispensers. The liquid soap used in these dispensers can become contaminated regardless of the preservative used when the microbial population exceeds the preservatives defenses. When product contamination has been reported, contamination was more likely to have occurred extrinsically (after product had been used) than intrinsically (during manufacturing). The likelihood of extrinsic contamination is greatest when the product is open to repeated exposure to bacteria from the user or the environment, hence, the packaging and the dispensing method plays a significant role in product safety.

Materials and Methods

Liquid soap samples were collected from public restrooms in five cities [Boston, MA (107), Atlanta, GA (120), Columbus, OH (109), Los Angeles, CA (94), and Dallas, TX (111)]. Samples were organized into 5 categories: office, health clubs, food service, retail locations and other (education, leisure, etc.). The total number of liquid soap samples analyzed in this report were 541, consisting of 428 soap samples from the sink area and 113 soap samples from the shower area at health clubs, 65 from men's showers and 48 from women's showers. A total of 428 liquid soap samples from the sink area, 226 from men's restroom sink areas and

202 from women's restroom sink areas, were analyzed for this report. Samples with <500 CFU/mL were not considered since industry standards allow for this amount of bacteria in liquid soap. All samples were confirmed to be from open refillable systems.

The samples were collected in sterile 50 mL conical tubes and shipped to the laboratory on ice. 1 mL of DE neutralizing broth (Remel, Lenexa, KS) was added to each sample tube and shaken vigorously for 60 seconds. Heterotrophic plate counts (HPC) were obtained by the spread plate method on R2A media (Difco, Sparks, MD). Plates were incubated at 30°C for 5 days. Any sample showing bacterial content was reexamined for Coliform bacteria.

Coliform analysis and enumeration was performed using the spread plate method on mEndo agar (Difco, Sparks, MD) and incubated at 35°C for 24 hours. Bacterial colonies were counted and recorded, representatives of all colony types were subcultured to TSA plates (Difco, Sparks, MD) for oxidase tests and identification. TSA plates were incubated at 35°C for 24 hours. Identification of bacteria was obtained using API20E strips (BioMerieux, Marcy-l'Étoile, France). *S. aureus* analysis was performed by using the spread plate method on TSA amended with 5% Sheep Blood (BA) (Hardy Diagnostics, Phoenix, AZ) to check for hemolysis. Plates were incubated for 24-48 hours at 35°C. Beta hemolytic isolates were enumerated and streaked onto a TSA plate and incubated for 24 hours at 35°C. Isolated colonies underwent further confirmation testing utilizing catalase production, microscopic morphology, coagulase production (tube and slide tests) and antibiotic (polymyxin) sensitivity.

Results

Figure 1: Locations Containing HCP and Percent of HCPs that were Coliforms

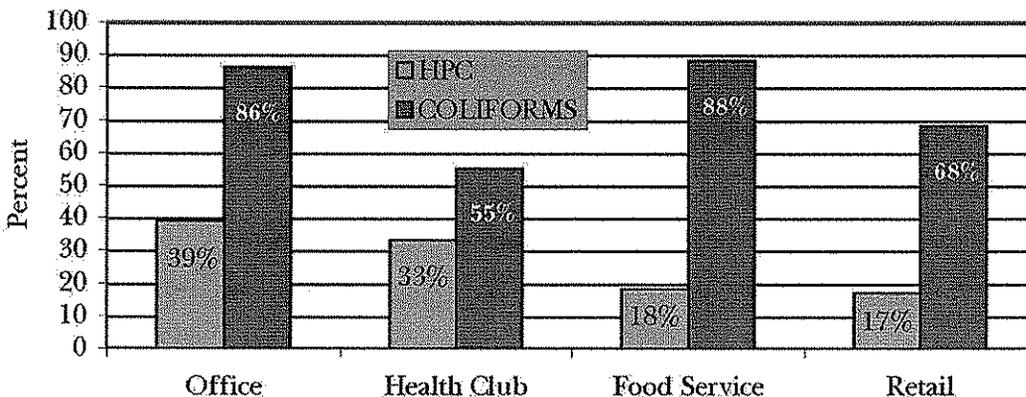


Figure 2: Frequency of Bacterial Species Isolated from Refillable Liquid Soap Dispensers

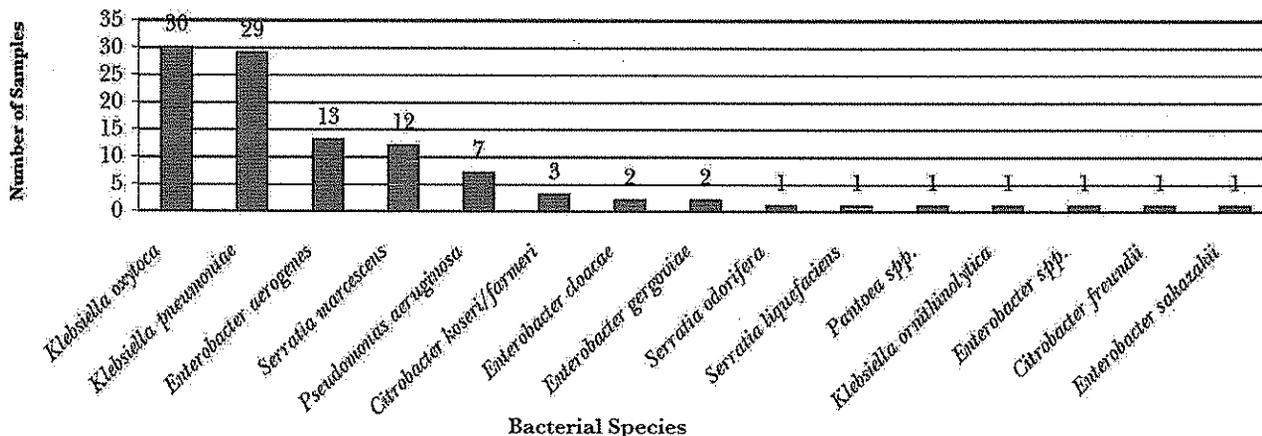
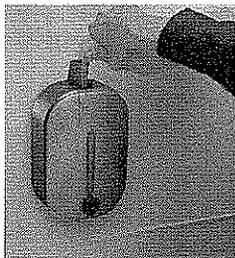


Figure 3:



Sealed System
0% Contaminated



Open Refillable Bulk
Soap Dispenser
Being Refilled
25% Contaminated

Table 1:

| Total number of open refillable soap samples | Number of samples with bacteria | Number of samples with Coliforms |
|--|---------------------------------|----------------------------------|
| 541 | 133 (25%) | 87 (16%) |

Summary

A total of 541 open refillable liquid soap samples were analyzed for bacteria, coliforms and *Staphylococcus aureus*. Of the 541 samples, 133 (25%) contained bacteria, 87 samples (16%) contained coliforms. The percent of bacteria isolated from open refillable liquid soap samples that were identified as coliforms was 65%. Heterotrophic bacterial numbers detected in the liquid soap samples ranged from 590 to 5.3×10^7 CFU/mL. The average number of bacteria found in one mL of soap was 3.02×10^6 CFU/mL. Coliform bacteria ranged from <10 to 6.5×10^7 CFU/mL, with an average of 3.94×10^6 per mL of soap. The frequency of contamination was similar for all cities tested, for both men and women's restrooms and for both wall mounted and counter-mounted dispensers. *Klebsiella* was the most frequently isolated genus of bacteria, followed by *Enterobacter* and *Serratia*. No *Staphylococcus aureus* were detected in any of the liquid soap samples analyzed.

Conclusions

High levels of bacterial contamination (average 3.02×10^6 CFU/mL) were found in 25% of the liquid soap samples in this study. Previous reports found no contamination in soap from sealed systems (figure 3). Since these samples represent a diverse cross section of geographical locales and individual sites, it is concluded that refillable open, or "bulk", liquid soap systems commonly found in the U.S. are routinely contaminated with bacteria. Many of the bacteria isolated are opportunistic pathogens which can cause a variety of health issues including respiratory infections, bloodstream infections, urinary tract infections and skin infections. The type and level of bacteria found in these systems represent a potential health risk to users, especially to any immunocompromised individuals.

Footnotes

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INTRODUCTION

Bulk refillable soap dispensers are manually refilled with bulk soap through an opening in the top, Figure 1.

Previous research demonstrated that up to 25% of bulk hand soap dispensers are contaminated with approximately 6 LOG₁₀(CFU/mL) heterotrophic bacteria based upon samples collected from the bulk soap¹. The contamination results from extrinsic sources and occurs when the preservative system in the soap is overcome.

This poster presents the results of a two-phase project. The goal of Phase 1 was to determine if biofilm growth within the dispensers contributed to bulk soap contamination, and Phase 2 investigated if washing the dispensers effectively reduced bacterial contamination.



Figure 1. Bulk refillable soap dispenser

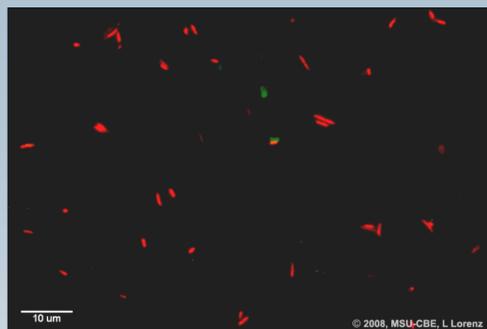


Figure 3. Epifluorescent image of cells obtained from a bulk refillable soap dispenser, filtered onto a polycarbonate membrane, and stained with Live/Dead for total cell counts. Total cell counts were an important way of determining the efficacy of the disaggregation methods. Disaggregation was determined to be efficient when single cells were seen, as shown above. 100X

Community Analysis Molecular Methods

The community analysis approach was broken down into four steps:

- Biomass collection**
 - Collect pooled bulk and surface associated pellets
- DNA preparation**
 - Cell lysis
 - Removal of cell debris via centrifugation
 - Precipitate proteins
- Clone library construction**
 - Clone gene of interest (SSU rRNA gene via PCR)
 - Ligation into plasmid & transformation into *E. coli*
 - Screen/pick colonies
- Organism identification**
 - Sequencing
 - Bioinformatic analysis

PHASE 1 – BIOFILM TESTING

Viable plate counts paired with biochemical identification assays and molecular methods were used to determine the amount of biofilm present and the ecology of the biofilm communities found in three types of dispensers. The dispenser types tested were: plastic counter-mount (from a shopping center), plastic wall-mount (from an elementary school), and stainless steel wall-mount (from middle/high schools). All dispensers tested were previously determined to be contaminated in the field.



Figure 2. Stereoscope image of dried soap on the spigot opening of a plastic wall-mount dispenser. 5X



Figures 4 and 5. Stereoscope images of unknown brown material found in all types of dispensers studied. Shown here: internal tubing from a counter-mounted dispenser (top) and lid of a plastic wall-mounted dispenser (bottom). 7.5X

BIOFILM TESTING RESULTS

Results indicated that (Figure 6) :

- The bulk soap, Sample A, was contaminated with 4-7 LOG₁₀(CFU/mL) bacteria.
- Samples B (loosely surface associated) and C (surface associated) contained 4-7 LOG₁₀(CFU/cm²), (n=6).
- Total cell counts ranged from 4-8 LOG₁₀(CFU/cm²) for all dispensers and sample types.

These results were Independent of dispenser type or construction material.

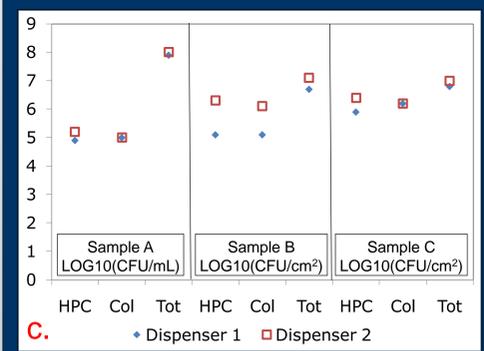
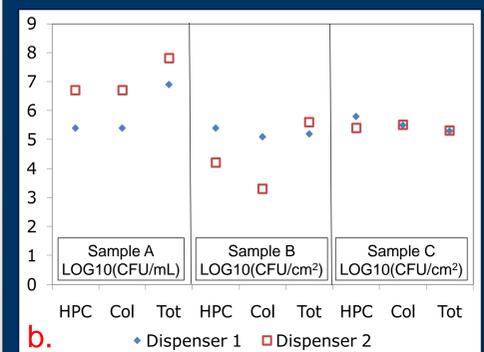
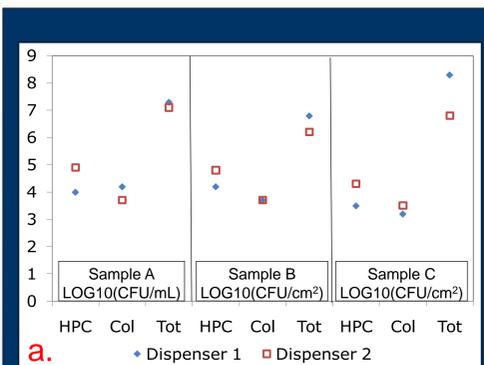


Figure 6. Panel a. is the counter-mounted dispenser results, b. the plastic wall-mounted dispenser results, and c. the stainless steel wall-mount dispenser results. For the viable plate count results, HPC refers to heterotrophic plate counts, Col refers to coliform counts and Tot refers to total cell counts. Samples A, B and C depict the bulk soap, loosely surface associate, and the surface associated biofilm counts, respectively.

METHODS COMPARISON

Overall, the results of the bacterial identification based upon biochemical assays versus molecular methods were comparable at the genus level, but some differences were observed (Table 1).

The biochemical profiling from all dispensers tested identified :

- 14 unique bacterial species
- 11 different genera

Whereas the molecular methods identified :

- 13 unique genera
- Possibly dozens of different species

All microorganisms observed are considered opportunistic pathogens and are mostly gram negative. The organisms identified were surprisingly consistent, and were independent of type and location of dispenser.

Table 1. Panel a. is an indirect comparison of field versus biochemically isolated microbes identified from the plastic wall-mounted dispensers. Panel b. is an indirect comparison of field identified microbes versus microbes identified using molecular methods from the plastic wall-mounted dispensers. Panel c. is a direct comparison of microbes identified using biochemical assays versus molecular based methods from stainless steel wall-mounted dispensers. Molecular ID isolates were based on DNA found in the dispensers, thus, viability of identified organisms could not be assessed.

| Dispenser # | Field ID | Biochemical ID | Molecular ID |
|-------------|---|--|--------------|
| 1 | <i>Providencia rettgeri</i> | <i>Providencia rettgeri</i> | |
| | <i>Pseudomonas aeruginosa</i> | <i>Pseudomonas aeruginosa</i> | |
| | <i>Citrobacter koseri</i> | <i>Serratia liquefaciens</i> | |
| | <i>Serratia odorifera</i> | <i>Klebsiella pneumoniae</i> | |
| 2 | <i>Pseudomonas aeruginosa</i> | <i>Pseudomonas aeruginosa</i> | |
| | <i>Stenotrophomonas maltophilia</i> | <i>Burkholderia cepacia</i> | |
| | <i>Aeromonas hydrophila</i> | Yeast, not <i>C. albicans</i> | |
| | | Gram positive bacillus (no further ID available) | |
| 3 | <i>Pseudomonas aeruginosa</i> | <i>Pseudomonas aeruginosa</i> | |
| | <i>Providencia rettgeri</i> | <i>Providencia rettgeri</i> | |
| | <i>Serratia rubidaea</i> | <i>Achromobacter xylosoxidans</i> | |
| | | <i>Alicyclopes xylooxidans</i> | |
| 4 | <i>Stenotrophomonas maltophilia</i> | <i>Stenotrophomonas sp.</i> | |
| | <i>Pseudomonas fluorescens</i> | <i>Pseudomonas aeruginosa</i> | |
| | <i>Pseudomonas luteola</i> | <i>Citrobacter sp.</i> | |
| | <i>Pseudomonas stutzeri</i> | <i>Enterobacter sp.</i> | |
| 22 | <i>Pseudomonas sp. – probably P. aeruginosa</i> | <i>Pseudomonas aeruginosa</i> | |
| | <i>Providencia sp. – probably P. rettgeri</i> | <i>Providencia/Proteus rettgeri</i> | |
| | <i>Serratia sp. – probably S. liquefaciens</i> | <i>Serratia marcescens</i> | |
| | <i>Providencia sp.</i> | <i>Providencia/Proteus rettgeri</i> | |
| 40 | <i>Stenotrophomonas sp.</i> | <i>Stenotrophomonas maltophilia</i> | |
| | <i>Pseudomonas sp.</i> | <i>Pseudomonas fluorescens/putida</i> | |
| | <i>Serratia sp.</i> | <i>Serratia liquefaciens</i> | |
| | <i>Alicyclopes/Achromobacter sp.</i> | <i>Acinetobacter lwoffii</i> | |

PHASE 2 – DISPENSER WASHING STUDY

Washing studies were completed to determine if dispensers could be washed or sanitized to eliminate future contamination. The methods used were selected to mimic options that could be available during routine restroom maintenance by janitorial staff. Three washing procedures were analyzed for plastic wall mounted bulk refillable soap dispensers:

- 1) a simple hot water rinsing technique
- 2) a hot water rinsing and scrubbing technique
- 3) a hot water rinse, scrub, 5,000mg/L bleach treatment, hot water rinse combination

Positive and negative control dispensers were drained and refilled with sterile soap.

Samples were collected from the rinse steps and evaluated for heterotrophic and coliform plate counts. Bulk soap sampling was performed for up to two weeks to determine washing procedure efficacy.



Figure 7. Stereoscope image of a fly found in the bottom dispenser assembly of a plastic wall-mounted dispenser. 7.5X

WASHING STUDY RESULTS

The washing study results (Figures 8-9) showed that bacterial counts in the bulk soap returned to pre-wash levels within two weeks of cleaning a dispenser and subsequently rinsing it with 5,000 mg/L bleach. The purple and blue X symbols represent the positive and negative control results, respectively.

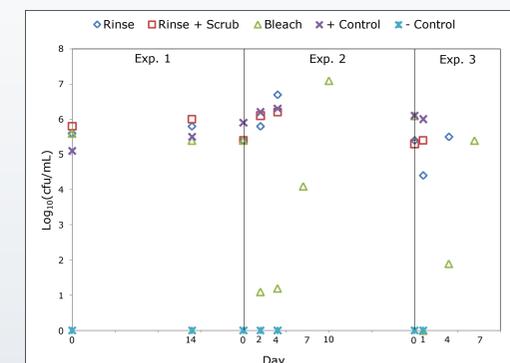


Figure 8. Dispenser washing study results: coliform counts

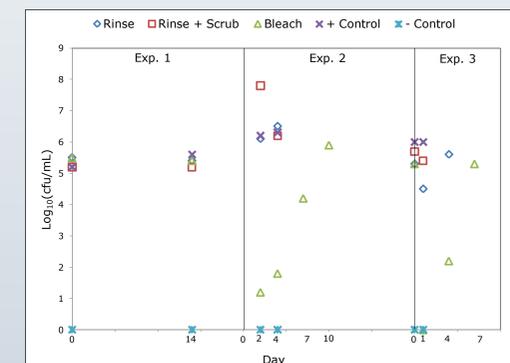


Figure 9. Dispenser washing study results: heterotrophic plate counts

CONCLUSIONS

- Dispensers contaminated with bacteria in the bulk soap also had high levels of biofilm bacteria.
- While the bacterial diversity was relatively low compared to other environments, detection of SSU rRNA gene sequences suggested the presence of organisms not detected via cultivation-based techniques (for some samples).
- The washing study results showed that bacterial counts in the bulk soap returned to pre-wash levels within two weeks regardless of the washing procedure used, although the bacterial counts in the dispensers rinsed with bleach did recover more slowly.

ACKNOWLEDGEMENTS

Funding was provided by GOJO Industries, Inc., Akron, OH.



¹Gerba CP, and Maxwell SL, "Bacterial contamination of liquid hand soaps used in public restrooms," Poster Presentation at NEHA 71st Annual Educational Conference & Exhibition, Atlantic City, NJ, 2007.



Handwashing with Contaminated Soap Results in Hand Contamination and Transfer of Bacteria



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Abstract

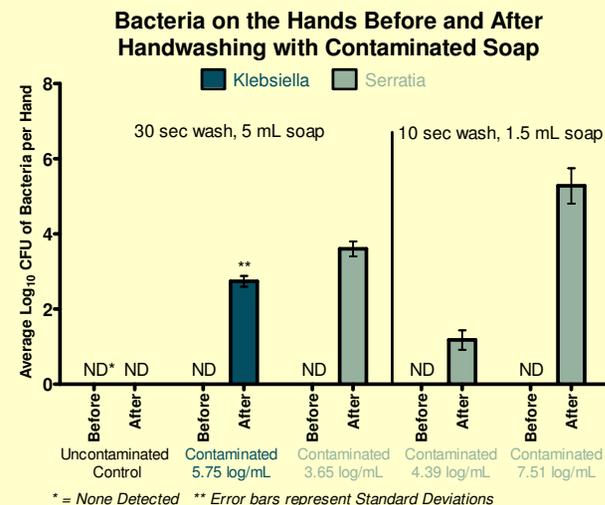
BACKGROUND/OBJECTIVE: Previous studies establish that open refillable bulk soap dispensers are often contaminated with species of *Klebsiella* and/or *Serratia* bacteria. In this study we evaluate whether these bacteria remain on the hands after handwashing and assess whether they can be transferred to other surfaces.
METHODS: Hands were sampled using the glove juice method before and after handwashing with contaminated or uncontaminated soap. In addition, some participants touched an agar surface. **RESULTS:** No *Klebsiella* or *Serratia* were detected on the hands before using the test soaps or after using the uncontaminated control soap. Between 15 and over 190,000 of the marker bacteria remained on each hand exposed to the contaminated soaps and the transfer of the bacteria was visible on the agar touch plates. **CONCLUSIONS:** Use of contaminated soap may contribute to the transmission of opportunistic pathogens such as *Klebsiella pneumoniae* and *Serratia marcescens*.

Methods

A laboratory simulation of handwashing with contaminated bulk soap was conducted. The testing methods were based on a modification of the FDA Tentative Final Monograph (TFM) for Effectiveness Testing of an Antiseptic Handwash or Health Care Personnel Handwash (FR59:116, 17 June 1994, pp. 31448-31450). Soap formulation chemistry, bacteria used, and levels of soap contamination tested simulated contaminated bulk soap found in public rest rooms. Two different handwash methods were tested. In the first study the handwash was designed to mimic an ideal procedure, e.g. one conducted by a healthcare worker (5 mL of soap, 30 sec wash, 30 sec rinse). In the second study the handwash was modeled after the typical washing behavior observed in the general public (1.5 mL of soap, 10 sec wash, 10 sec rinse). A total of 5 soap samples were tested; one uncontaminated control, one sample contaminated with *Klebsiella pneumoniae*, and three samples contaminated with *Serratia marcescens*. *Klebsiella* and *Serratia* were used since they were two of the most common types of bacteria found in contaminated bulk soap, accounting for over 2/3 of all contaminants. Contaminated samples were prepared by repeatedly inoculating unpreserved soap formulations with bacteria until the soap became contaminated. A range of levels of contamination were tested from relatively low (<10,000 CFU/mL, <4 Log₁₀CFU/mL) to high (>10,000,000 CFU/mL, >7 Log₁₀CFU/mL) bacterial contamination. The number of contaminating bacteria on both hands of 6 participants were measured before and after handwashing with each test soap (N=12) using the glove juice method. In addition, after washing with each soap 1 or 2 participants touched an agar surface with one or both of their hands (N=2 or N=4).

Results

- ✓ None of the participants had detectable amounts of *Klebsiella* or *Serratia* on their hands before washing
- ✓ No *Klebsiella* or *Serratia* were detected on hands after washing with an uncontaminated control soap
- ✓ After handwashing with contaminated soap between 15 to over 190,000 bacteria from the soap remained on each hand (averaging from 1.18 to 5.28 Log₁₀ CFU).
- ✓ Use of soap with the highest contamination level resulted in the greatest contamination level on the hands.



- ✓ Both *Klebsiella* and *Serratia* from the contaminated hands of participants were transferred to agar surfaces following handwashing with contaminated soap.



Background



Bulk



Sealed

Permanently mounted soap dispensers provided in public restrooms can be refilled either with sealed cartridges/bags or by pouring soap from a larger bulk container such as a gallon jug. Since soap contaminated with bacteria has been linked to outbreaks, the CDC recommends against the use of bulk soap dispensers in healthcare settings. However, in non-healthcare settings, bulk dispensers are still quite common and are often contaminated. Recent reports have found that 23-25% of open refillable bulk soap dispensers found in public restrooms are contaminated with unsafe levels of potentially pathogenic organisms. Sealed dispensing systems were free from contamination. With a growing immunocompromised population, it is prudent to investigate how remediation of this unnecessary health risk could reduce the risk of community-acquired infections. The objective of this study was to evaluate whether bacteria from contaminated soap remains on the hands after handwashing and to assess whether they can be transferred to other surfaces.

Conclusions

- ✓ Washing hands with contaminated soap results in contamination of the hands and transfer of the bacteria to surfaces.
- ✓ Contaminated bulk soap may contribute to the transmission of opportunistic pathogens such as *Klebsiella* and *Serratia*.
- ✓ Further research is needed to evaluate the public health risk of using contaminated bulk soap by patrons of public restrooms.

Opportunistic Pathogens From Contaminated Bulk Soap on the Hands of Students and Staff in an Elementary School

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Abstract

Previous research revealed that approximately 23% of open refillable bulk soap dispensers in public restrooms are contaminated with an average of 3,000,000 bacteria/ml soap. This study was performed to evaluate hand contamination and bacterial transmission by hands after washing with bulk soap. Gram-negative bacteria on the hands of 10 students and 10 staff were quantified before and after using either contaminated bulk soap or uncontaminated control soap. In addition, the transfer of gram-negative bacteria from the hands to an agar surface was evaluated. Hands were found to harbor over 10-fold more opportunistic pathogens after washing with contaminated bulk soap than before washing (2047 vs 179). An average of 1 gram-negative bacterium was transferred to surfaces touched by students or staff either before the hand wash or after washing with uncontaminated control soap. After washing with the contaminated soap, the average number of gram-negative bacteria transferred to surfaces increased to 38 for children and 9 for adults. These results suggest that contaminated bulk soap may play a role in the transmission of bacteria in schools, particularly among children.

Background

Hand soap dispensers used in school restrooms can be refilled with soap that is either bulk or sealed. Bulk dispensers are refilled by pouring soap from a large container into the open reservoir and typically the nozzle that the soap is ejected through is not replaced. In contrast, sealed dispensers are refilled by replacing bags or cartridges that contain soap sealed inside with a new nozzle. Soap in bulk dispensers is prone to contamination because it is constantly exposed to bacteria from the environment, such as from the hands of the person refilling the soap, the spray of toilet water after flushing, or from dust in the air. Since contaminated bulk soap dispensers have caused outbreaks in hospitals, the CDC recommends against their use in healthcare settings. However, no such guidelines exist to protect patrons of public restrooms in the community or our students in schools. In our previous studies, we tested soap from over 500 dispensers across the United States to evaluate the prevalence of contaminated soap in public restrooms. We were surprised to learn that 1 in 4 bulk dispensers are contaminated with an average of over 3 million bacteria, most of which are known to be opportunistic pathogens. Exposure to such high levels of these organisms can be a significant health risk to individuals with compromised immune systems which is estimated to be at least 20% of the population. In contrast, soap from sealed dispensing systems was free from contamination. We identified an elementary school in which the antibacterial soap in all of their plastic wall mounted bulk soap dispensers were highly contaminated with 19 different species of *Pseudomonas*, *Providencia*, *Citrobacter*, *Stenotrophomonas*, *Aeromonas*, *Enterobacter*, *Pasteurella*, and *Serratia* bacteria. The objective of this study was to evaluate bacterial hand contamination and hand transmission among children and adults in an elementary school with a contaminated bulk soap problem.

Methods

10 staff and 10 students each participated in up to 4 handwashes each using one of 14 contaminated bulk soap dispensers. 11 staff participated in up to 2 handwashes each during the follow up study which was conducted 4 months after the contaminated bulk soap dispensers were replaced with sealed soap dispensing systems. Participants were instructed to wash and dry their hands as they normally would after using the restroom. All hands were tested both before and after handwashing using one of two methods.



Method A: The number of bacteria on one hand of each participant was measured using the glove juice procedure.



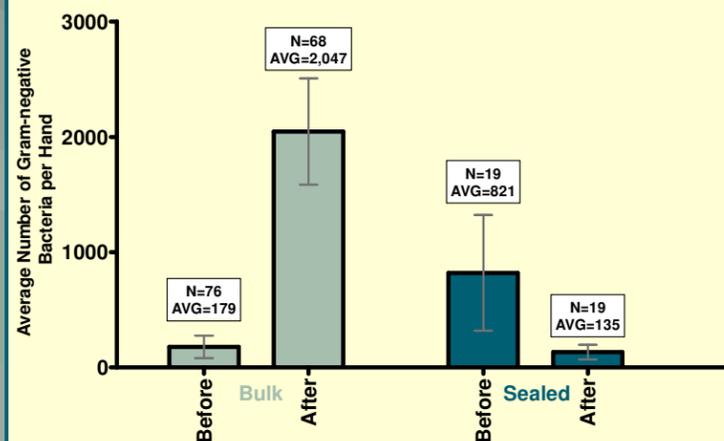
Method B: Bacterial transfer to a surface was measured with the opposite hand using the hand stamp procedure.



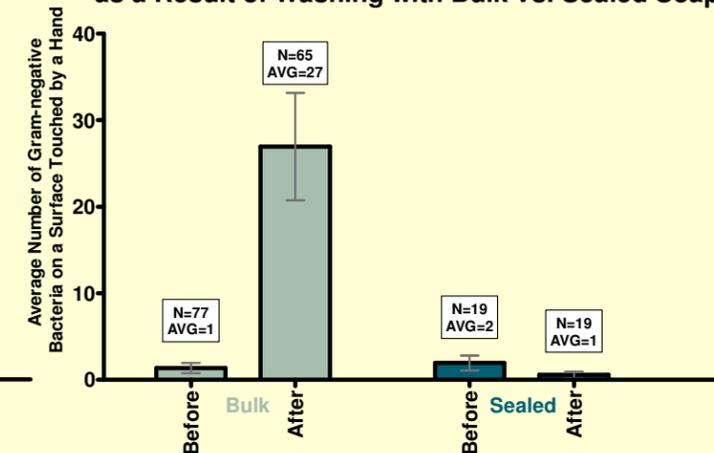
Results were obtained from counting bacteria that grew on MacConkey's agar. Statistical comparisons were performed using the Student's t-test on the Log₁₀ transformed bacterial colony counts.

Results

A) Relative Effectiveness of Handwashing with Contaminated Bulk vs. Sealed Soap



B) Bacteria Transferred by Hands to a Surface as a Result of Washing with Bulk vs. Sealed Soap



- ✓ Washing with contaminated bulk soap significantly increased the number of gram-negative bacteria per hand from 179 to 2047 on average for all students and staff ($P < 0.0001$). Students' hands retained significantly more bacteria than the staff, 3148 vs. 474 ($P < 0.01$).
- ✓ Washing with sealed soap significantly reduced the number of bacteria from 821 to 135 ($P < 0.05$).
- ✓ Hands had significantly less gram-negative bacteria after washing with sealed soap compared to after washing with contaminated bulk soap, 135 vs. 2047 ($P < 0.0001$).
- ✓ Washing with contaminated bulk soap significantly increased the number of gram-negative bacteria transferred to a surface from 1 before washing to 27 after on average for all students and staff ($P < 0.0001$). Students transferred significantly more bacteria to the surface they touched after washing with contaminated bulk soap than the staff did, 38 vs. 9 ($P < 0.01$).

Conclusions

- ✓ Hand soap dispensers which are refilled by pouring bulk soap into an open reservoir are often contaminated with opportunistic pathogens.
- ✓ Washing with contaminated bulk soap resulted in a 10-fold increase in the number of pathogenic bacteria that were found on the hands of students and staff in an elementary school.
- ✓ Hands washed with contaminated bulk soap transferred a significantly higher number of opportunistic pathogens to touched surfaces compared to hands washed with soap from a sealed refill.
- ✓ Contaminated bulk soap may play a role in the transmission of bacteria in schools, particularly among children.
- ✓ Schools using bulk soap dispensers could reduce the potential risk of infections by upgrading to dispensers which utilize only sealed soap refills.

Open Refillable Bulk Soap Dispensers in Public Restrooms: A Public Health Risk?

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Presenter Disclosures

Dr. Charles P. Gerba

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GOJO Industries, Inc.
Sponsored Research

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Learning Objectives

1. Describe how to identify open refillable bulk soap dispensers
2. Explain why open refillable soap dispensers are susceptible to bacterial contamination
3. Discuss why contaminated bulk soap in community settings could be a public health risk, particularly for susceptible populations

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Soap Microbial Quality

- Handwash products are regulated by the FDA. Excessive contamination is a violation.¹
- Soap is not expected to be sterile.
 - “It is the responsibility of the manufacturer to assure that... the species and quantity of microbes do not present a hazard to the consumer when using the product as directed...”²
- Guidelines recommend <1000 total bacteria/mL & the absence of pathogens.²

1) <http://www.cfsan.fda.gov/~dms/cos-218.html>
 2) The Cosmetic, Toiletry, and Fragrance Association, Technical Guidelines, Microbial Limits for Cosmetics and Toiletries, 2001
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Soap Dispensers

- Open Refillable Bulk
 - Refilled by pouring soap from a larger volume container
 - Open to environment
 - Same nozzle used indefinitely
- Closed Sealed Systems
 - Soap provided in a disposable sealed bag or cartridge refill
 - New nozzle with each refill



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How Does the Soap Become Contaminated?



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Contaminated Soap Causes Infection in Health-Care Settings

- Many reported infections and outbreaks¹
 - Fatal *Pseudomonas aeruginosa* infection from use of contaminated shampoo²
 - *Serratia marcescens* infections linked to contaminated soap. Hands 54 times more likely to be contaminated after washing³
- Susceptible populations are at greatest risk
 - >20% of US population is immune-compromised⁴

1) Weber D, Rutala V, and Sickbert-Bennett E. Antimicrobial Agents and Chemotherapy 2007 Dec;51(12):4217-4224.
2) Fainstein V, Andres N, Umphrey J, and Hopfer R. J. Infect. Dis. 158:655, 1988.
3) Santor C, Jacome V, Duwiver C et al. Infect. Control Hosp. Epidemiol. 2000 March;21(3):196-9.
4) Gerba, D, Rose, J, Haas C. International Journal of Food Microbiology 30 (1996) 113-123.
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CDC Health-Care Recommendation

- “Do not add soap to a partially empty soap dispenser. This practice of “topping off” dispensers can lead to bacterial contamination of soap¹.”
- Since the risk is well-documented bulk dispensers are rare in Health-Care

1) Guideline for Hand Hygiene in Health-Care Settings, Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Morbidity and Mortality Weekly Report, October 30, 2002 / Vol. 51 / No. 88-14.
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Is it Safe to Use Bulk Soap Dispensers in Community Settings?

- Prior to our research, no studies had been conducted in the US to assess this potential risk.
- Our studies indicate that patrons of public restrooms are routinely exposed to unsafe levels of bacterial contamination.
- This represents an unnecessary health risk, particularly for the immunocompromised susceptible population.

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Bulk Soap Contamination is Widespread

- Soap from over 500 bulk dispensers in public restrooms were tested from across the US
- Unsafe levels of bacteria occur in 23 - 25% of bulk soap dispensers^{1,2}
 - Fecal-based organisms found in over 16% of the soap samples
 - Average user exposed to >one million bacteria per handwash
 - Soap from sealed systems showed no contamination

1 M. Chattman, S. Maxwell and C. P. Gerba; Bacterial Contamination of Liquid Hand Soaps, University of Arizona, Tucson, AZ, American Society for Microbiology 107th General Meeting, Toronto, ON, Canada, May 21-25, 2007.
2 C. P. Gerba and S. Maxwell, University of Arizona, Tucson, AZ; National Environmental Health Association 71st Annual Educational Conference & Exhibition, Atlantic City, NJ; June 18-21, 2007.
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Bacteria Remain on Hands After Washing with Contaminated Soap

- Bacterial contaminants remain on hands after handwashing and are transferred to touched surfaces^{1,2}



After washing with liquid soap that was not contaminated



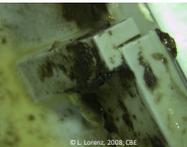
After washing with contaminated liquid soap

1 BioScience Laboratories, Inc.; Bozeman, MT; Study #071209-150; Feb 22, 2008.
2 BioScience Laboratories, Inc.; Bozeman, MT; Study #080307-150; May 22, 2008.
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Contaminated Dispensers Should Be Replaced, Cleaning Is Ineffective

- New soap is re-contaminated by biofilm bacteria adhering to inside of dispenser
- Even dispensers scrubbed with hot water and sanitized with 5000 mg/L bleach were contaminated 7-10 days after new uncontaminated soap was added





Lorenz et al. Evaluation of Contaminated Bulk Soap Dispensers for Biofilm Bacteria; Comparison of Two Methods of Analysis and Effectiveness of Dispenser Washing Procedures. Montana State University Center for Biofilm Engineering Poster to be presented at the 5th ASM Conference on Biofilms, Nov 2009.
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Study Objectives

- Assess the factors contributing to contamination
 - Are some types of soap more likely to become contaminated?
 - Are certain types/models of bulk dispensers more susceptible to contamination?
 - How do contamination rates compare between different types of facilities?
- Test for the presence of specific organisms of public health concern
 - Food-borne pathogen *E. coli*
 - Antibiotic-resistant organisms

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Method- Soap Sampling

- ~ 10 mL of soap collected into sterile collection containers and tested <1 week
- 155 bulk samples collected from Ohio
 - restaurants, bars, gas stations, schools, office buildings, retail stores, health clubs, grocery stores, theaters, etc.



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Method- Soap Analysis

- Microbial load
 - Dilute into buffer with neutralizers and plate onto R2A
 - >1000 CFU/mL threshold for contamination
 - Dominant colony types identified
- Active ingredient
 - HPLC used to determine % PCMX or Triclosan
- Food-borne pathogen screen
 - Enrichment based water quality test used to determine if *Escherichia coli* bacteria were present
- Antibiotic resistance
 - Contaminants were tested for their ability to grow on media containing antibiotics, two classes were tested

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Results- High levels of Contamination Observed

- 21% (32/155) of bulk soap samples were contaminated with >1000 CFU/mL bacteria
- Average level 6.3×10^6 CFU/mL
- 13 different gram negative species isolated including *Pseudomonas*, *Providencia*, *Achromobacter*, *Citrobacter* and *Serratia*
 - These opportunistic pathogens can cause respiratory tract infections, pneumonia, urinary tract infections, pink eye, skin ulcers, gastroenteritis, soft tissue infections, etc.

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Results- All Types of Bulk Dispensers from All Types of Facilities Were Contaminated

| Type of Facility | Total | Contaminated | % |
|------------------|-------|--------------|-----|
| Shopping | 22 | 4 | 18% |
| Recreation | 15 | 3 | 20% |
| Dining | 28 | 6 | 21% |
| Other/Unknown | 90 | 19 | 21% |

| Dispenser Type | Total | Contaminated | % |
|----------------|-------|--------------|-----|
| Counter | 21 | 3 | 14% |
| Wall plastic | 48 | 8 | 17% |
| Wall metal | 16 | 4 | 25% |
| Other/Unknown | 70 | 17 | 24% |

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Results- All Types of Soap Were Contaminated

| Type of Soap | Total | Contaminated | % |
|--------------------------|-------|--------------|-----|
| Bland | 110 | 23 | 21% |
| Antimicrobial- Triclosan | 26 | 8 | 31% |
| Antimicrobial- PCMX | 14 | 1 | 7% |
| Other/Unknown | 5 | 0 | 0% |

| Color of Soap | Total | Contaminated | % |
|---------------|-------|--------------|-----|
| Blue | 6 | 1 | 17% |
| clear/white | 33 | 2 | 6% |
| green | 13 | 4 | 31% |
| orange | 31 | 12 | 39% |
| pink | 55 | 11 | 20% |
| peach | 9 | 0 | 0% |
| yellow | 6 | 2 | 33% |
| Other/Unknown | 2 | 0 | 0% |

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Results- *E. coli* and Antibiotic Resistant Bacteria Were Found

- *E. coli* was detectable in 28% (7/25) of the contaminated soaps tested
- Resistance to quinolones or ceftazidime was observed in 28% (22/78) of the isolates, most frequently in species of *Pseudomonas*, 68% (15/22), but also in *Klebsiella*, *Serratia*, *Burkholderia* and *Enterobacter* species.
 - 5% (4/78) of the isolates were resistant to both antibiotics.

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Conclusions

- Bulk hand soap is prone to bacterial contamination.
- Contamination is associated with the open design of the dispenser.
 - it is not limited to any particular type of soap or type of bulk dispenser
- Contaminated soap can harbor food-borne pathogens and antibiotic resistant organisms.

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Conclusions

- The species typically found in contaminated soap can cause infections.
- Immune-compromised handwashers with poor skin integrity are at greatest risk of acquiring an infection.
- Further research is warranted to determine the extent to which contaminated bulk soap in public restrooms poses an unnecessary public health risk.

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What Can You Do?

- Notice what type of soap dispensers are used in the areas you service
- Educate facilities about the potential risk
- If reoccurring infections due to gram negative pathogens occur, consider testing the soap as a possible reservoir
- Particularly in settings with high proportions of susceptible patrons, recommend the use of sealed systems

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 - Esther Campbell
- Montana State University Center for Biofilm Engineering
 - Dr. Darla Goeres
 - Lindsey Lorenz
 - Dr. Matthew Fields
 - Brad Ramsay

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Thank You

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APHA 137th Annual Meeting and Expo: November 7-11, 2009; Philadelphia, Pennsylvania

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Issue Attachment:

Elimination of Open, Refillable Soap Dispensers

Health Canada

"Guidance Document: Human-Use Antiseptic Drugs" Effective 11/27/2009

Page 33/34 Section 7.4 Labelling

"Do not refill container."

http://www.hc-sc.gc.ca/dhp-mps/alt_formats/pdf/prodpharma/applic-demande/guide-ld/antiseptic_guide_ld-eng.pdf