UNIVERSITY OF ARIZONA STUDY

THE OCCURRENCE OF HETEROTROPHIC BACTERIA, COLIFORMS AND *STAPHYLOCOCCUS AUREUS* IN LIQUID SOAP SAMPLES FROM PUBLIC RESTROOMS

Charles P. Gerba, Sheri L. Maxwell,
Dept. of Soil, Water, and Environmental Science,
The University of Arizona, Tucson, AZ
January 23, 2006

Conclusion

High levels of bacterial contamination (average 3.02x106CFU/mL) were found in 25% of the liquid soap samples in this study. 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. The type and level of bacteria found in these systems represent a potential health risk to users, especially to any immunocompromised individuals.

Objective

The objective of this study was to determine the occurrence of heterotrophic bacteria, coliforms and *Staphylococcus aureus* in liquid hand soaps collected from public restrooms across the country. The identification of coliform and non-coliform bacteria detected in soap samples was also determined. The liquid soap samples collected were from refillable dispensers (also referred to as "open systems" or "bulk soap" systems). A total of 541 liquid soap samples from sinks and showers were used for this project.

Summary

A total of 541 liquid soap samples were analyzed for bacteria, coliforms and *Staphylococcus aureus.* Of the 541 samples, 135 (25%) had bacteria, 86 samples (16%) contained coliforms. The average number of bacteria was 3.02xl06 CFU/mL with a range of 590 to 5.3xl07 CFU/mL. The average number of coliforms was 3.94xl06 CFU/mL with a range of <10 to 6.5xl07 CFU/mL. No *Staphylococcus aureus* was detected.

Results

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 showers. Results showing number of bacteria and Coliforms in 1 mL of liquid soap samples are shown in

Table 1. Samples with <500 colony forming units (CFU)/mL were not considered since industry standards allow for this amount of bacteria in liquid soap.
Table 1. Number of samples with heterotrophic bacteria (HPC) and Coliforms
In table 2, the percent of liquid soap samples that contained HPC bacteria and Coliforms are shown.

Table 2. Percent of samples with HPC and Coliforms
Heterotrophic bacterial numbers detected in the liquid soap samples ranged from 590 to 5.3x107CFU/mL. The average number of bacteria found in 1mL of soap was 3.02x106. Coliform bacteria ranged from <10 to 6.5x107CFU/mL in liquid soap samples, with an average of 3.94x106per mL of soap. No *Staphylococcus aureus* were detected in any of the liquid soap samples analyzed.
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, was analyzed for this report. Results of the number and percent ofHPC bacteria and coliforms detected in the soap samples from the sink area are found in Table 3.

Total Number of Number of Samples Number of Samples
Liquid Soap Samples with HPC with Coliforms
541 135 86
Total Number of Liquid Percent of Samples with Percent of Samples with
Soap Samples HPC Coliforms
541 25% 16%

Table 3. Number of samples with HPC and Coliforms detected from soap in sink area including a breakdown of men and women restrooms. Percent of samples with HPC and Coliforms also shown. A total of 113 liquid soap samples were from the shower area at fitness centers or health clubs, 65 from men's showers and 48 samples were from women's showers. Results of the number and percent ofHPC bacteria and Coliforms detected in soap samples from the shower area are found in Table 4.

Table 4. Number of samples with HPC and Coliforms found in soap samples in the shower area including an analysis of men and women showers. Percent of samples with HPC and Coliforms are also shown. Bacterial identification and frequency of isolation in soap samples can be found in Table 5.

Table 5. Identification and frequency of bacteria isolated from soap samples.
Total Number of Number of Number of Percent of Percent of
Soaps Sampled Samples Samples with Samples Samples with
in Sink Area with HPC Coliforms with HPC Coliforms
428 85 55 20% 13%
Men
Restroom 226 41 24 18% 11%
Women
Restroom 202 44 31 22% 15%
Total Number of Number of Number of Percent of Percent of
Soap Sampled in Samples Samples with Samples Samples with
Shower Area with HPC Coliforms with HPC Coliforms
113 50 38 44% 34%
Men
Shower 65 31 27 48% 42%
Women.

Shower 48 19 11 40% 23%
Bacteria Frequency of Isolation
Klebsiella oxytoca 30
Klebsiella pneumoniae 29
Enterobacter aerogenes 13
Serratia marcescens 13
Pseudomonas aeruginosa 8
Citrobacterfreundii 5
Citrobacter koseri/farmeri 3
Enterobacter cloacae 2
Enterobacter sakazakii 1
Enterobacter eroviae 1
Serratia odori(era 1
Serratia lique(aciens 1
Pantoea spp. 1
Klebsiella ornithinolytica 1

Materials and Methods

Liquid soap samples were collected from public restrooms in five cities (Boston, MA, Atlanta, GA, Columbus, OH, Los Angeles, CA, and Dallas, TX). Approximately 20% of the individual sampling sites were independently audited after 30 days. All samples were confirmed to be from refillable systems.

The samples were collected in sterile 50 mL conical tubes and shipped to the laboratory on ice. 1 mL 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 spread plating 0.1 mL of sample onto duplicate R2A plates (Difco, Sparks, MD), plates were then incubated at 30°C for 5 days. After incubation, colonies were counted and the mean reported. Samples with plates containing colonies that were too numerous to count were enumerated from original sample by ten-fold dilutions in sterile buffered saline and replated on duplicate R2A plates. Any sample showing bacterial content was reexamined for Coliform and *Staphylococcus aureus* bacteria.

Coliform analysis and enumeration was performed by spread plating the appropriate dilution of the original sample on mEndo agar plates (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. pxidase tests were done on all colony isolates and results used in conjunction with bacterial identification. Oxidase tests were performed by applying Kovacs reagent (Hardy Diagnostics, Phoenix, AZ) to filter paper to which isolated colonies were applied using a sterile platinum loop. A positive reaction is characterized by a deep purple color within 10 seconds; a negative reaction is indicated by the absence of color in the same time. Positive control *(Pseudomonas aeruginosa* ATCC# 27313) and negative control *(Escherichia coli* ATCC# 25597) were run concurrently to ensure quality of Kovacs reagent. Identification of bacteria was obtained using API20E strips (BioMerieux, Marcy-I'Etoile, France).

S. *aureus* analysis was performed by spread plating the appropriate dilution of the original sample 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.