APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1984, p. 338–341
0099-2240/84/080338-04$02.00/0

Vol. 48, No. 2

Microbial Flora of In-Use Soap Products

MOLLIE E. McBRIDE

Department of Dermatology, Baylor College of Medicine, Houston, Texas 77030

Received 29 December 1983/Accepted 4 May 1984

A comparison has been made of the in-use bacterial load of two bar soaps with and without antibacterials and two liquid soaps in five different locations over a 1-week period. Of the 25 samples taken from each soap, 92 to 96% of samples from bar soaps were culture positive as compared to 8% of those from liquid soaps. Bacterial populations ranged from 0 to 3.8 log CFU per sample for bar soaps and from 0 to 2.0 log CFU per sample for liquid soaps. The mean bacterial populations per sample were 1.96 and 2.47 log CFU for the two bar soaps, and 0.08 and 0.12 log CFU for the two liquid soaps. The difference in bacterial population between bar soaps and liquid soaps was statistically significant ($P = 0.005$). *Staphylococcus aureus* was isolated on three occasions from bar soaps but not from liquid soaps. *S. aureus* was isolated twice from the exterior of the plastic dispensers of liquid soap but not from the soap itself. Gram-negative bacteria were cultured only from soaps containing antibacterials. Bacterial populations on bar soaps were not high compared with bacterial populations on hands, and the flora was continually changing without evidence of a carrier state.

Hand washing continues to be the single most important step in the prevention of the spread of infection in hospitals, but its importance is equally great in the food industry and in the home. There is a wealth of information on the antimicrobial properties of soaps, detergents, and disinfectants and their efficacy for the removal of microorganisms from skin (6). There have also been reports of contamination of disinfectants and cleaning solutions in hospitals, leading to outbreaks of infection (2, 4, 10, 11). In 1965, a study on bar soaps artificially inoculated with bacteria revealed a self-disinfecting activity (1), but the actual level of microbial exposure encountered in day-to-day hand washing with over-the-counter soap products has not been evaluated. The purpose of this study was to determine the numbers and types of micoorganisms that are present in soap products while the products are in use and to compare bar soaps with liquid soaps in plastic disposable containers.

MATERIALS AND METHODS

Two bar soaps, Ivory (Proctor & Gamble, Cincinnatti, Ohio), without antibacterials, and Dial (Armour-Dial, Phoenix, Ariz.), containing the antibacterial Triclocarban (Mansanto, England), were compared with two liquid soaps, Softsoap and Showermate (Minnetonka, Inc., Minnetonka, Minn.). Showermate contains an antibacterial, Triclosan (Ciba-Gelgy, United Kingdom), and Softsoap contains a preservative, DMDM hydantoin. Bacterial populations were determined quantitatively and qualitatively from each product over a 7-day period in five different locations. Three of these sites were two washrooms and a hand-washing station in the microbiology laboratory in a clinical department which served ca. 30 people, including physicians, scientists, and laboratory and administrative personnel. The two remaining test sites were in a dermatology clinic: a washroom used by physicians, nurses, patients, and office personnel and a nurses' hand-washing station. Hand washers were asked to record each use of the soaps, and each product was weighed before and after the 1-week trial period to ensure adequate use. Products were sampled at the same time each day (1:00 p.m.) before use (0 time) and after 1, 2, 3, 4, and 7 days of use, making a total of 30 samples per product. On day 7, the exterior of the plastic dispenser was also sampled. Bar soap receptacles permitted drainage, and at all sampling times bar soaps were moist but not wet.

The sampling method chosen was designed to approximate the amount of soap used during a normal hand-washing procedure. In the case of liquid soap, one squirt from the dispenser provided enough soap for an adequate hand-washing procedure; the dispensing device delivered uniformly very close to 0.9 ml of liquid soap; therefore, this was the amount chosen for sampling. Estimating the amount of bar soap used during one hand washing is more difficult, and hence a procedure was devised whereby the bar was placed in a plastic bag containing 10 ml of sampling liquid. The bar was massaged in liquid for 15 s as one would in a normal hand-washing procedure. The bar was then removed and returned for use. In each case the amount of soap used for a normal hand-washing procedure was suspended in 10 ml of phosphate buffer (pH 7.2) containing 2% polysorbate 80 (Tween 80; BBL Microbiology Systems) as a neutralizer. The outside surfaces of the liquid soap containers were sampled by immersing the upper half of the container into a plastic bag containing 10 ml of the same buffer-Tween 80 mixture. Polysorbate 80 is commonly used as a neutralizer and is one of the ingredients of D-E Neutralizing Medium (Difco Laboratories) (3). Preliminary tests were done to evaluate 2% Tween 80 as a neutralizing agent for the antibacterials in these soap products. A quantitated inoculum of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Serratia marcescens* was added to the soap samples taken as described above. Bacterial counts of the soap samples with neutralizer were equivalent to those in buffer alone and in buffer with 2% Tween 80.

For quantitation of the samples, 0.1 ml was inoculated on the surfaces of four plates of tryptic soy agar (Difco) containing 2% Tween 80. Duplicate plates were incubated aerobically at 35°C for 3 days and in a ANEE anaerobic incubator for up to 7 days to obtain duplicate counts of aerobes and anaerobes. A 1.0-ml portion of the sample was also inoculated into 9 ml of thiogycolate broth containing 2% Tween 80 and incubated for 24 h in the event that the bacterial populations were too low to be detected in the 0.1-ml portion used for plate inoculations. In these cases, by calculations, the population in the 10-ml sample would be between 10 and 99 bacteria, or log 1. Fewer than 10 bacteria

TABLE 1. Bacterial populations of soap products tested in five locations

Location[a]	Day of sampling	Bacterial population (log CFU per 10-ml sample) of[b]:			
		Ivory	Dial	Soft-soap	Shower-mate
FN	1	3.0	2.7	1.0	ND[c]
	2	2.0	2.7	ND	ND
	3	2.0	2.7	ND	ND
	4	3.8	3.7	ND	ND
	7	2.9	2.3	ND	ND
FS	1	1.0	2.6	ND	ND
	2	ND	3.3	ND	1.0
	3	2.6	2.4	ND	ND
	4	2.0	2.2	ND	ND
	7	1.6	3.2	1.0	ND
SN	1	2.2	2.0	ND	ND
	2	2.0	1.7	ND	2.0
	3	1.0	1.5	ND	ND
	4	2.3	ND	ND	ND
	7	2.2	1.6	ND	ND
SB	1	2.9	2.9	ND	ND
	2	2.4	2.0	ND	ND
	3	2.9	3.4	ND	ND
	4	2.5	2.2	ND	ND
	7	3.0	3.0	ND	ND
S11	1	1.0	2.3	ND	ND
	2	1.7	2.3	ND	ND
	3	1.0	2.8	ND	ND
	4	ND	3.8	ND	ND
	7	1.0	2.5	ND	ND

[a] FN and FS, Washrooms in clinical department; S11, hand-washing station in the microbiology laboratory of the same department; SB, washroom in dermatology clinic; SN, nurses' hand-washing station in the same clinic.

[b] For Ivory, mean, 1.96; standard deviation, 0.95; variance, 0.87; for Dial, mean, 2.47; standard deviation, 0.79; variance, 0.06; for Softsoap, mean, 0.08; standard deviation, 0.28; variance, 0.07; for Showermate, mean, 0.12; standard deviation, 0.12; variance, 0.19.

[c] ND, None detected.

per 10-ml sample could not be detected by this method. After incubation, 0.1 ml of thioglycolate broth was subcultured to Casman sheep blood agar and incubated for 24 h. Populations were calculated and expressed as CFU per 10-ml sample and were compared statistically by using Student's *t* test. Mycosel (BBL) agar was also inoculated with 0.1 ml of the sampling liquid and incubated for 3 weeks for the isolation of fungi.

For qualitative analysis of samples, Casman sheep blood agar was inoculated with 0.1 ml of sampling liquid, and plates were streaked for isolation. All isolates demonstrating colonially distinct morphology were identified. Common air- and skin-normal flora such as *Bacillus* spp. and coryneforms were identified at the genus level only. All medically important organisms were identified at the genus and species level by standard methodology (5), supplemented by the API Staph Ident system and the API Anaerobic system. Yeasts were identified by the Minitek system (BBL).

RESULTS

Neither bacteria nor fungi were isolated from any of the products before use. After use, microorganisms were isolated from Ivory in 23 of the 25 samples and from Dial in 24 of 25 samples, as compared with isolations in 2 of 25 samples for each of the liquid soaps. The data on bacterial populations from each product, expressed as log CFU per 10-ml sample, are shown in Table 1. On three of the four occasions when bacteria were detected in liquid soap, bacteria were present only in the thioglycolate enrichment in populations of less than 100. Although the bacterial populations on Ivory were found to be lower than those on Dial, the difference was not found to be statistically significant ($P = 1.1$). The difference between the bacterial populations on bar soaps and those in either of the liquid soaps, however, was highly significant ($P = 0.005$).

The frequency of isolation of the different species of bacteria isolated from soap products is shown in Table 2. Species of *Staphylococcus* other than *S. aureus* were found to be the most prevalent and were present in 21 of 25 cultures from Ivory and in 24 of 25 cultures from Dial. *Staphylococcus aureus* was the principal medically important organism isolated on two occasions from Ivory in populations of 300 and less than 100 and once from Dial in a population of 350. The only enteric gram-negative rod, *Escherichia coli*, was isolated from Showermate on a single occasion in a population of 100. Other gram-negative organisms found were isolated from Dial at a single sampling period and were *Acinetobacter calcoaceticus*, *Flavobacterium odoratum*, and another *Flavobacterium* sp. The pattern of isolation for all species was found to be random. Organisms would appear and disappear from day to day, indicating some form of self-disinfecting activity or, in the case of bar soaps, mechanical removal. The isolation of *E. coli* from Showermate on day 2 of the trial was followed by a sterile sample on day 3, and this was the pattern of isolation from all samples for all organisms, including *Staphylococcus aureus*.

Staphylococcal species were identified with the API Staph Ident system to determine if there was a pattern of carriage which could be related to either the soap or the location. These results are summarized in Fig. 1. It can be seen that the isolation of any particular species was random.

Fungi were not isolated from liquid soaps. Species cultured from bar soaps were *Candida parapsilosis*, *Aspergillis niger*, *Nocardia* spp., *Aspergillis candidus*, *Streptomyces* spp., and *Penicillium* spp., none of which represent significant pathogens. Isolation of fungi was sporadic, was not identified with a specific location, and was in character with air contamination.

Anaerobic bacteria were not a prominant part of the flora. *Propionibacterium acnes* was isolated once from Ivory and twice from Dial. A species of *Eubacterium* and *Peptococcus*

TABLE 2. Frequency of isolation of aerobic bacteria from 25 samples of soap products taken over a 7-day period

Organism	No. of samples positive			
	Ivory	Dial	Softsoap	Showermate
Staphylococcus spp. (other than *Staphylococcus aureus*)	21	24	1	1
Staphylococcus aureus	2	1	0	0
Bacillus spp.	5	5	0	1
Coryneforms	6	3	0	0
Micrococcus spp.	3	5	0	0
Gram-negative rods	0	3	0	1
Nocardia spp.	1	0	0	0
Streptomyces spp.	0	1	0	0

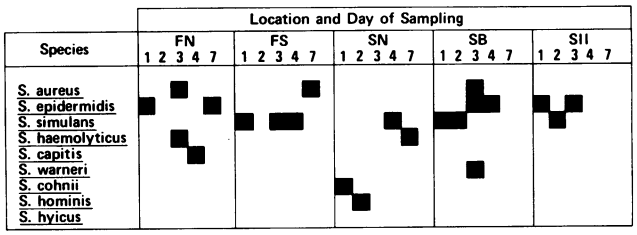

FIG. 1. Distribution of *Staphylococcus* species on Ivory soap during use for 1 week in five locations. Abbreviations for locations are defined in Table 1, footnote *a*.

saccharolyticus were isolated from Dial, each on a single occasion.

Populations of bacteria on liquid soap dispensers (Table 3) ranged from 2.0 to 3.0 log CFU per sample from Softsoap and from 2.0 to 4.0 log CFU per sample from Showermate. Certain test locations were predisposed to higher populations, although contamination of the soap did not correlate with this. The types of microorganisms found on the containers are shown in Table 4. The organisms isolated were predominantly staphylococcal species other than *Staphylococcus aureus* and part of the spectrum of normal skin flora. *Staphylococcus aureus* was isolated twice from containers of Softsoap without contamination of the soap contents.

DISCUSSION

The main aim of this study was to evaluate the microbial flora of soap products in an in-use environment. Depature from a controlled laboratory protocol introduces a number of variables, one of which is the question of the role of hydration on bacterial proliferation on bar soaps. It was debated whether bar soaps should be sampled in their wet or dry state, since it is logical to assume that wet bars would support higher microbial populations. In a preliminary trial, no obvious differences were found between wet and dry bars; hence, a sampling time was chosen (midday) which would ensure that the product had been used for several hours; therefore, bar soaps were always moist when sampled. Liquid soaps were found to be relatively free of bacterial contamination, which is no doubt due to the fact that liquid soap can be dispensed without direct exposure to skin bacteria. Although the dispenser can be heavily populated by microorganisms, these do not appear to gain entrance. On the other hand, bar soaps are in direct contact with bacteria on skin, and organisms were found to survive on bar soaps which were continually in use. The self-sterilizing activity described by Bannan and Judge (1) in a laboratory-controlled study does not appear to occur in the continual-use situation. The reasons for this are probably multiple, but it is logical to assume that the bar soaps in use are continually being reinoculated by bacteria from hands. As well, bacteria deposited on the surface of soap from hands may be suspended in organic matter which would protect viability. Many substances are known to function in this way, from sugar solutions to complex proteins such as serum and other organic compounds (12).

Compared with bacterial populations on hands, which may range up to 10^5 organisms per cm^2 of skin (9), the numbers of bacteria found on bar soaps were not large. Furthermore, the populations did not progressively increase throughout the 1-week test period, indicating that the organisms were continually being removed, either by self-sterilization or mechanically. This possibility is also supported by the qualitative data, since a carrier state did not exist, either on bars or in liquid soap. *Staphylococcus aureus* appeared sporadically and disappeared spontaneously. Similarly, the isolation of *E. coli* from liquid soap on one occasion was followed by a negative culture on the following day. Liquid soaps contain preservatives which no doubt contribute to this continually changing pattern of flora.

Soaps containing antibacterials were as susceptible to bacterial carriage as those without. It is interesting to note that the isolation of gram-negative bacteria was confined to soaps containing antibacterials. Gram-negative bacteria have been associated with contamination of disinfectant solutions, e.g., *Pseudomonas cepacia* (11) and *Serratia marcescens* (7) in chlorhexidine. Both povidone-iodine and benzalkonium solutions have also been reported as serving as a source of contamination (2, 4, 11). The term antibacterial, as used by the soap industry, is a broad one, and little information is available on the spectrum of the antibacterials contained in soaps. The active ingredient listed in Dial is Triclocarban, and that in Showermate is Triclosan. Both have been described as broad-spectrum antibacterials (8), but details of trials and effective concentrations have not been published in the scientific literature.

TABLE 3. Populations of bacteria on liquid soap dispensers

Location[a]	Bacterial population on dispenser for:	
	Softsoap	Showermate
SN	500	650
SB	150	650
S11	750	750
FN	2,500	1,200
FS	3,100	10,500

[a] Abbreviations are defined in Table 1, footnote *a*.

TABLE 4. Microorganisms isolated from liquid soap dispensers

Location[a]	Microorganisms isolated from dispenser for:	
	Softsoap	Showermate
SN	*Staphylococcus aureus*	*Staphylococcus epidermidis* Coryneforms *Propionibacterium acnes*
SB	*Staphylococcus aureus* *Staphylococcus warneri* *Staphylococcus epidermidis*	*Staphylococcus epidermidis* *Bacillus* spp. *Staphylococcus epidermidis* *Propionibacterium acnes*
S11	*Staphylococcus capitis* *Staphylococcus epidermidis* Coryneforms	*Staphylococcus epidermidis*
FN	*Staphylococcus simulans* *Propionibacterium acnes* Coryneforms	*Staphylococcus epidermidis* *Micrococcus* spp.
FS	Coryneforms *Aspergillis niger*	*Staphylococcus epidermidis* Coryneforms

[a] Abbreviations are defined in Table 1, footnote *a*.

ACKNOWLEDGMENTS

This work was supported by a grant from Minnetonka, Inc.

The technical assistance of Douglas Troll is gratefully acknowledged, and the excellent cooperation of all the personnel in the Department of Dermatology, Baylor College of Medicine, while the study was in progress is greatly appreciated.

LITERATURE CITED

1. **Bannan, E. A., and L. F. Judge.** 1965. Bacteriological studies relating to handwashing. Am. J. Public Health **55**:915–922.
2. **Berkelman, R. L., S. Levin, J. R. Allen, R. L. Anderson, L. D. Budnick, S. Shapiro, S. M. Freidman, P. Nicholas, R. S. Holzman, and R. W. Haley.** 1981. Pseudobacteremia attributed to contamination of povidone-iodine with *Pseudomonas cepacia*. Ann. Intern. Med. **95**:32–36.
3. **Engley, F. B., and B. P. Dey.** 1970. Universal neutralizing medium for antimicrobial chemicals. Chem. Spec. Manuf. Assoc. Proc. **56**:100–106.
4. **Fox, J. G., C. M. Beaucage, C. A. Folta, and G. W. Thornton.** 1981. Nosocomial transmission of *Serratia marcescens* in a veterinary hospital due to contamination by benzalkonium chloride. J. Clin. Microbiol. **14**:152–160.
5. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.).** 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
6. **Maibach, H., and R. Aly.** 1981. Skin microbiology: relevance to clinical infection. Springer Verlag, New York.
7. **Marrie, T. J., and J. W. Costerton.** 1981. Prolonged survival of *Serratia marcescens* in chlorhexidine. Appl. Environ. Microbiol. **42**:1093–1102.
8. **Marzulli, F. N., and M. Bruch.** 1981. Antimicrobial soaps: benefit vs. risks, p. 125–133. *In* H. Maibach and R. Aly (ed.), Skin microbiology: relevance to clinical infection. Springer Verlag, New York.
9. **McBride, M. E., L. F. Montes, W. J. Fahlberg, and J. M. Knox.** 1972. Microbial flora of nurses hands. I. Quantitative differences in bacterial population between nurses and other occupational groups. Int. J. Dermatol. **11**:49–53.
10. **Sheets, R. D.** 1981. *Serratia marcescens* contaminated disinfectants. Lancet **i**:727.
11. **Sobel, J. D., N. Hashman, G. Reinberz, and D. Merzbach.** 1982. Nosocomial *Pseudomonas cepacia* infection associated with chlorhexidine contamination. Am. J. Med. **73**:183–186.
12. **Ulrich, J. A.** 1981. Antimicrobial efficacy in the presence of organic matter, p. 149–157. *In* H. Maibach and R. Aly (ed.), Skin microbiology: relevance to clinical infection. Springer Verlag, New York.