APPLIED MICROBIOLOGY, Mar. 1973, p. 431-435
Vol. 25, No. 3

Printed in U.S.A.

Survival of Microorganisms in Laundered Polyester-Cotton Sheeting[1]

JOANNE CLARQUIST WIKSELL,[2] MARY S. PICKETT, AND PAUL A. HARTMAN
Departments of Family Environment and Bacteriology, Iowa State University, Ames, Iowa 50010

Received for publication 6 November 1972

The effects of wash-water temperature, cold-water or regular detergent, wash-cycle design, drying, and drying temperature on survival of four microorganisms on polyester-cotton sheeting were examined. *Escherichia coli* T3 bacteriophage survived washing at 24, 35, 46, and 57 C, but not at 68 C. *Serratia marcescens* survived only the lowest three wash temperatures. Levels of residual *Staphylococcus aureus* were diminished at the highest two wash temperatures, but survival was substantial even at 68 C. Counts of *Bacillus stearothermophilus* spores were not altered appreciably by wash temperature. Type of detergent had no practical effect on observed counts. The regular wash cycle was significantly more efficient in removal of microorganisms than the permanent-press cycle. Counts, especially of the bacteriophage and the gram-negative bacterium, were decreased by drying; after drying, the effects of wash-water temperature on *S. aureus* and *B. stearothermophilus* were not significantly different. Microorganisms were transferred from inoculated to sterilized sheeting during laundering. The public health significance of these observations is discussed.

Dissemination of microorganisms from fabrics to man can be brought about by bed-making, dressing, sorting laundry, and exercising (2, 5, 8, 11, 15, 18, 25). Certain pathogens also can persist through the laundering and drying processes. Some excellent studies have been conducted on the microbiology of laundering (3, 4, 6, 13, 14, 16, 17, 19, 20, 23), but these seem not to have attracted much public attention.

In the light of increased usage of self-service laundry facilities, there is reason for concern for nosocomial infections. Coupled with greater use of self-service laundry facilities is pressure to use greater quantities of cold water and cold-water detergents. The development of fabrics designed with basic characteristics whose maintenance depends upon lower wash-water and drying temperatures presents an additional problem of public health significance. Bed linens made of blends of new fibers are being used extensively where the characteristic of minimum wrinkling is associated with appearance. Possible lack of sanitation causes even more concern when bed linens are cared for on the premises of motels and hotels.

The chance of infection, especially for the more susceptible individual, is as great as the degree of exposure to potential pathogens. We examined, therefore, the effects of wash-water temperature, detergent type, wash-cycle design, drying, and drying temperature on survival of selected microorganisms on polyester-cotton sheeting.

MATERIALS AND METHODS

The T3 bacteriophage of *Escherichia coli* B was propagated according to the method described by Songer, Sullivan, and Hurd (22) and was stored at 4 C. *Serratia marcescens* 2G12 and *Staphylococcus aureus* Ps29 were maintained on Brain Heart Infusion (Difco) agar slants. Fabric inocula were prepared by transferring one loopful of cells from a slant into a 250-ml flask containing 85 ml of heart infusion broth (Difco), followed by incubation for 22 to 24 h at the optimal temperature for growth. *Bacillus stearothermophilus* ATCC 7954 spore preparations were made by a method similar to that of Finley and Fields (9); the spore suspensions were stored at 4 C.

An inoculum was prepared for each set of experiments by combining the following preparations in a 625-ml, wide-mouth bottle: 10 ml of T3 bacteriophage (9×10^8 plaque-forming units/ml), 85 ml each of *S. marcescens* and *S. aureus* cultures (3×10^8 to 4

[1] Journal Paper No. J-7307 of the Iowa Agriculture and Home Economics Experiment Station, Ames. Project No. 1807.

[2] Present address: Department of Home Economics, University of Northern Iowa, Cedar Falls, Iowa 50613.

$\times 10^8$ cells/ml), and 20 ml of a *B. stearothermophilus* spore suspension (5×10^7 spores/ml).

Squares of 50% polyester-50% cotton, permanent-press sheeting (23 by 23 cm) were conditioned by washing three times in hot water with a regular anionic detergent, wrapped in aluminum foil, and sterilized in an autoclave. Thirteen conditioned fabric samples were inoculated for each experiment by placing them individually in a bottle of inoculum. The bottle was inverted five times to achieve saturation of each sample with inoculum. The sample was then removed from the bottle dripping wet and allowed to air-dry for 2 h on a rack in a small room containing a commercial household dehumidifier. The room was equipped with ultraviolet lights, which were turned on between experiments to reduce the load of airborne microorganisms. Four fabric samples were used to determine counts of microorganisms before washing, and the remaining fabric samples were carried through various laundering and drying procedures.

Each wash load was composed of nine inoculated samples, three sterile samples, and a sterile cotton filler load to make a total of 1.8 kg. The 20-min permanent-press cycle of an automatic washer with gentle agitation (44 oscillations per min) and a slow spin (410 rpm) was used for washing some of the samples; other samples were washed by using the 35-min regular cycle (68 oscillations per min and 625 rpm spin). The volumes of water used were as follows: wash, 49 liters; cool-down, 34 liters; and cold rinse at 22 C, 76 liters. Unless specified otherwise, 160 g of a regular anionic detergent was added to 49 liters of water for each wash load. Initial wash temperatures of 24, 35, 46, 57, and 68 C were obtained by varying the ratios of hot to cold water. Two replications were made at each wash temperature. Temperatures were measured by immersing a mercury thermometer in the water. Residual microbial carry-over between washes was minimized by a hot-water wash cycle after each experiment.

Three inoculated samples were analyzed for microbial content undried after washing, three were dried in an automatic dryer with half of the filler load at a low drying temperature (46 C), and three were dried with the remainder of the filler load at a high drying temperature (60 C). The permanent-press drying cycle consisted of a 20-min heat phase and a 10-min cooling phase. The three "sterile" fabric samples were examined without drying.

Each fabric sample was placed in a 625-ml bottle containing 500 ml of sterile 0.1% peptone buffer (24) and shaken vigorously (150 times in 1 min); then serial dilutions appropriate for enumerating the test microorganisms were made in peptone buffer. T3 bacteriophage were titrated by the method of Adams (1). Counts of the three bacteria were made by surface-plating 0.1-ml quantities onto plates of a 50:50 mixture of antibiotic medium 2 (Difco) and neomycin assay agar (BBL). *S. marcescens* formed red colonies after incubation for 48 h at 25 C, *S. aureus* formed typical colonies after incubation for 48 h at 37 C, and *B. stearothermophilus* colonies were counted after incubation for 48 h at 55 C.

The logarithmic values of the treatment of means for each microorganism were used for the statistical analyses (21). Factorial designs with randomized blocks were used for the analysis of variance (J. C. Wiksell, M.S. Thesis, Iowa State Univ., Ames, 1971).

RESULTS

Counts of microorganisms (expressed as $\log_{10}$) recovered from fabric samples before washing, after washing at temperature of 24, 35, 46, 57, or 68 C, and after drying are shown in Table 1. The relatively low counts of T3 phage on the fabric after washing at 24, 35, and 46 C (Table 1) and the relatively high counts in the wash waters at these temperatures (Table 2) indicate that the virus particles adhere less tenaciously than the bacterial cells and spores to the fabric. A wash temperature of 57 C resulted in greater than 90% reduction in phage counts of the washed fabric, compared with lower wash temperatures. No *S. marcescens* cells were recovered from either fabric (Table 1) or wash water (Table 2) when an initial temperature of 57 C or higher was used. Drying at low temperature (46 C) resulted in almost complete destruction of the T3 and *S. marcescens* inocula (Table 1).

With *S. aureus*, increases in wash temperatures from 35 to 46 C and 46 to 57 C significantly reduced [0.01 level of probability (L.P.)] the number of viable cells recovered from the samples (Table 1). There was no significant difference, however, in counts of *S. aureus* made after use of wash temperatures of 57 and 68 C. The possibility of recontamination of the fabric cannot be ruled out because the counts were low, but this is unlikely because viable *S. aureus* cells also were recovered from the wash water at 68 C (Table 2). Drying at low temperature significantly reduced the numbers of viable *S. aureus* cells; nevertheless, some cells survived all washing and drying combinations (Table 1).

Significantly fewer spores were recovered from samples laundered at 46 and 57 C than from samples washed at 24 and 35 C (0.01 L. P.). Although this difference is sufficient to be statistically significant, it is of little practical importance because high spore counts were obtained from samples after washing at all temperatures. High spore counts also were obtained from all wash and rinse waters (Table 2) as well as from fabric samples after drying (Table 1).

Analyses of the counts of the four test organisms recovered from sheeting samples washed at the specified temperatures and samples dried at 46 C (Table 1) showed that a signifi-

cant reduction in counts was caused by drying (0.001 L.P.). Whether the lowered counts were due to inactivation or death of the microorganisms during drying or to lowered recovery because of greater adherence to the fabric after drying was not ascertained; we believe that both factors might be involved. There was, however, no significant effect due to initial wash-water temperature when the samples were dried after washing; only with *B. stearothermophilus* can any trend be noticed ("After drying," Table 1).

A comparison of low (46 C) versus high (60 C) drying temperature was confined to counts of *S. aureus* and *B. stearothermophilus* because few T3 phage and no *S. marcescens* cells survived even the low drying temperature. Data from all 10 washing procedures were combined for analysis. Geometric means (expressed as $\log_{10}$) of *S. aureus* cells and *B. stearothermophilus* spores surviving per square centimeter of sheeting were 0.529 and 2.995, respectively, after drying at low temperature, and 0.418 and 3.001, respectively, after drying at high temperatures. Thus, there were no significant differences in counts of the two microorganisms due to drying temperature.

Mean counts of the test microorganisms recovered from samples washed in cold (24 and 35 C) water with the permanent-press cycle and either a regular detergent or a cold-water detergent are shown in Table 3. Significantly fewer (0.025 L.P.) T3 phage were recovered from samples washed in a regular detergent (Table 3). Significantly fewer (0.05 L.P.) *S. aureus* cells were recovered from samples dried after washing with a cold-water detergent than from those dried after use of a regular detergent. These two differences, although statistically significant, are not of any practical importance. No other comparisons resulted in significant differences; therefore, we conclude that detergent type was a minor factor in affecting reduction of microbial loads on polyester-cotton sheeting.

The permanent-press and regular wash cycles were compared (Table 4) by using sheeting samples washed in cold (24 or 35 C) water. The regular wash cycle, which was longer and more vigorous than the permanent-press cycle, effected the greatest reduction in residual numbers of all four test microorganisms. The effects of wash-cycle design, however, could not be demonstrated after the samples were dried.

Infectious T3 bacteriophage particles and viable *S. marcescens* cells were recovered from sterilized samples washed with inoculated samples at some of the lower wash temperatures (Table 5), but the counts were low. The minimal degree of transfer of phage at low wash temperatures (Table 5) supports our contention that the phage do not readily adhere to the fabric. The *S. marcescens* counts for samples washed at 46 C could have been contaminants because a few colonies were observed on only 3 of 12 plates; otherwise, only the wash temperature of 24 C resulted in significant transfer of this gram-negative bacterium. Although the counts of *S. aureus* cells and *B. stearothermophilus* spores transferred to steri-

TABLE 1. *Geometric means ($\log_{10}$) of survivors per square centimeter on polyester-cotton sheeting before washing, after washing, and after washing and drying*[a]

Time of count	Wash temp (C)	T3 phage	*S. marcescens*	*S. aureus*	*B. stearothermophilus*
Before washing	—	4.41	5.19	5.52	4.68
After washing	24	1.83	3.84	3.77	3.95
	35	1.69	2.41	3.96	4.01
	46	1.34	1.01	2.45	3.34
	57	0.69	0.0	1.03	3.17
	68	0.0	0.0	1.20	2.97
After drying	24	0.24	0.0	0.54	3.17
	35	0.0	0.0	1.12	3.14
	46	0.0	0.0	0.84	2.74
	57	0.0	0.0	0.45	2.73
	68	0.0	0.0	0.63	2.45

[a] A regular detergent was used with the permanent-press wash cycle, and a low (40 C) drying temperature was used.

TABLE 2. *Arithmetic means ($\log_{10}$) of microorganisms recovered per milliliter of wash water at the end of the wash cycle and of rinse water at the end of the rinse cycle*[a]

Sample	Wash temp (C)	T3 phage	*S. marcescens*	*S. aureus*	*B. stearothermophilus*
Wash water	24	3.66	2.32	1.95	3.62
	35	3.08	1.48	1.54	3.36
	46	3.34	1.36	0.0	3.43
	57	1.30	0.0	0.70	3.54
	68	0.0	0.0	0.70	3.08
Rinse water	24	1.60	0.48	1.00	2.45
	35	0.90	0.0	1.36	2.46
	46	0.0	0.0	1.30	2.40
	57	0.0	0.0	1.36	2.43
	68	0.0	0.0	0.0	2.20

[a] Experimental conditions were the same as those listed for Table 1.

TABLE 3. *Geometric means (log_{10}) of survivors per square centimeter on polyester-cotton sheeting washed in cold water with regular and cold-water detergents*[a]

Time of count	Detergent	T3 phage	*S. marcescens*	*S. aureus*	*B. stearothermophilus*
After washing	Regular	1.76	3.13	3.86	3.98
	Cold water	1.13	3.56	3.45	4.08
After drying	Regular	0.12	0.0	0.83	3.16
	Cold water	0.0	0.0	0.27	3.45

[a] Data for two wash temperatures, 24 and 35 C, did not differ statistically and were combined. The permanent-press wash cycle was used. The pH values of the wash waters for the regular and cold-water detergents varied between 8.8 and 8.9.

TABLE 4. *Geometric means (log_{10}) of survivors per square centimeter on polyester-cotton sheeting washed in cold water with the permanent-press and regular wash cycles*[a]

Time of count	Wash cycle	T3 phage	*S. marcescens*	*S. aureus*	*B. stearothermophilus*
After washing	Permanent press	1.44	3.34	3.66	4.03
	Regular	0.43	2.84	2.65	3.32
After drying	Permanent press	0.06	0.0	0.55	3.31
	Regular	0.0	0.0	0.58	3.00

[a] Data for two wash temperatures, 24 and 35 C, did not differ statistically and were combined. A regular detergent was used.

TABLE 5. *Geometric means (log_{10}) of microorganisms recovered from sterilized samples of polyester-cotton sheeting that were washed with inoculated samples*[a]

Wash temp (C)	T3 phage	*S. marcescens*	*S. aureus*	*B. stearothermophilus*
24	0.24	1.12	1.48	1.45
35	0.0	0.0	1.10	1.13
46	0.56	0.81	1.23	0.56
57	0.0	0.0	1.28	0.63
68	0.0	0.0	0.54	0.45

[a] The permanent press cycle and a regular detergent were used.

lized samples during washing were low (Table 5), they demonstrate that the laundering process can result in cross-contamination from one article to another.

DISCUSSION

The actual effect of wash-water temperature in the destruction of microorganisms on fabrics during laundering is difficult to determine accurately because the observed reduction in counts of microorganisms could be due solely to removal and dilution of the microorganisms (19). We used relatively large numbers of test microorganisms to inoculate the fabric samples, so that recovery under at least some of the test circumstances would be assured. The large numbers of *B. stearothermophilus* spores and other microorganisms recovered from the wash and rinse waters (Table 2) indicate that physical removal of the microorganisms occurred. These data are similar to work reported by Sidwell et al. (19). Detergents might also increase physical removal by lowering the surface tension (19); however, no samples were washed without detergent in our study, so the relative effects of detergent omission are not known.

Results of this study showed that wash temperatures and cycle design had an effect on the numbers of microorganisms recoverable from fabric samples after washing. These results are in agreement with previous studies (13, 19).

Although results of this study showed that wash temperature and cycle affected the survival of the test microorganisms, drying in an automatic dryer obscured the wash-temperature and cycle effects. Sidwell et al. (19) reported that, when samples were stored for 20 h after washing, few or no poliovirus were detectable; the samples had dried during this period. It was suggested that possible alterations of the chemical structure of the virus or of the surroundings made the virus more labile than normal.

Our results agree with those of others (14, 20) who have reported that microorganisms are transferred during laundering to sterilized samples placed in the same load. Our data show that gram-positive bacteria can be transferred to uncontaminated fabrics during laundering at all wash-water temperatures commonly used in household washing machines. These results were not unexpected, but they emphasize the importance of being aware of the vastly greater resistance of these bacteria compared with viruses and gram-negative bacteria.

The possibility of dissemination of viruses and *Enterobacteriaceae* within a family by laundering processes can be controlled by the homemaker by washing clothing or bedding of an ill person separately and by using all methods that contribute to the removal and destruction of microorganisms. These methods include washing in the hottest permissible or available temperature and using a long, vigorous cycle, followed by drying in an automatic dryer. These procedures can be followed easily when the homemaker owns the equipment. Dependence on communal facilities usually means that there is much less control over water temperature and, because of a set cost for individual loads, there may be a tendency to combine the clothing or bedding of an infected person with that of the other family members. When communal facilities are used, dissemination of pathogenic microorganisms within a family would seem secondary to dissemination between families. The results of several investigators (10, 12) indicate that microorganisms can be disseminated not only in the same load but also between loads and between the families who use communal facilities. Thus, the use of all procedures contributing to sanitation are necessary, especially for communal facilities. It would also seem to be essential to laundering of linens from motels and hotels, where the numbers and pathogenicity of contaminants is unknown. Even then, vigorous washing in hot water and drying will not assure that the laundry is freed from pathogens.

The homemaker may want to choose cold water and a short, gentle wash cycle for laundering some materials. These conditions are specified on the labels of many fabrics. The homemaker, however, takes a calculated risk that the laundry contains no pathogens capable of surviving the washing and drying processes. This risk can be decreased by use of longer wash cycles and hotter water. Additional treatment, such as use of a bactericide in the wash or rinse water, are necessary if the risk is to be reduced even further. Possible recontamination of the laundered goods after drying (7) is an additional problem.

ACKNOWLEDGMENTS

Richard Warren provided help in analysis of the data and Teryl K. Frey provided laboratory assistance.

LITERATURE CITED

1. Adams, M. H. 1950. Methods of study of bacterial viruses. Methods Med. Res. **2**:1-73.
2. Anderson, K. F., and R. A. W. Sheppard. 1959. Dissemination of *Staphyloccoccus aureus* from woollen blankets. Lancet **1**:514-515.
3. Anonymous. 1969. Tests prove no-iron linens sanitary. Amer. Dyest. Rep. **58**(13):29.
4. Arnold, L. 1938. A sanitary study of commercial laundry practices. Amer. J. Pub. Health **28**:839-844.
5. Charnley, J., and N. Eftekhar. 1969. Penetration of gown material by organisms from the surgeon's body. Lancet **1**:172-174.
6. Church, B. D., and C. G. Loosli. 1953. The role of the laundry in the recontamination of washed bedding. J. Infec. Dis. **93**:65-74.
7. Consumer and Food Economics Research Division, Agricultural Research Service. 1967. Sanitation in home laundering (revised edition). U.S. Dep. Agr. Home Gard. Bull. **97**:1-8.
8. Downie, A. W., M. Meiklejohn, L. St. Vincent, A. R. Rao, B. V. S. Babu, and C. H. Kempe. 1965. The recovery of smallpox virus from patients and their environment in a smallpox hospital. Bull. W.H.O. **33**:615-622.
9. Finley, N., and M. L. Fields. 1962. Heat activation and heat-induced dormancy of *Bacillus stearothermophilus* spores. Appl. Microbiol. **10**:231-236.
10. Kundsin, R. B., C. W. Walter, J. Ipsen, and M. D. Brubaker. 1963. Ecology of staphylococcal disease. J. Amer. Med. Ass. **185**:159-162.
11. Litsky, B. Y., and W. Litsky. 1971. Bacterial shedding during bed-stripping of reusable and disposable linens as detected by the high volume air sampler. Health Lab. Sci. **8**:29-34.
12. McNeil, E. 1963. Studies of bacteria isolated from home laundering. Develop. Ind. Microbiol. **4**:314-318.
13. McNeil, E. 1964. Dissemination of microorganisms by fabrics and leather. Develop. Ind. Microbiol. **5**:30-35.
14. McNeil, E., and E. A. Choper. 1962. Disinfectants in home laundering. Soap Chem. Spec. **38**(8):51-54, 94-100.
15. McNeil, E., and M. Greenstein. 1961. Control of transmission of bacteria by textiles and clothing. Chem. Spec. Mfr. Ass. Proc., p. 134-141.
16. Meyers, J. R. 1968. Bacteriological study shows short-time, low temperature washing procedure inadequate. Linen Supply News, June.
17. Nicholes, P. S. 1970. Bacteria in laundered fabrics. Amer. J. Pub. Health **60**:2175-2180.
18. Sciple, G. W., D. K. Riemensnider, and C. A. J. Schleyer. 1967. Recovery of microorganisms shed by humans into a sterilized environment. Appl. Microbiol. **15**:1388-1392.
19. Sidwell, R. W., G. J. Dixon, L. Westbrook, and F. H. Forziati. 1971. Quantitative studies on fabrics as disseminators of viruses. V. Effect of laundering on poliovirus-contaminated fabrics. Appl. Microbiol. **21**:227-234.
20. Sidwell, R. W., L. Westbrook, G. J. Dixon, and W. F. Happich. 1970. Potentially infectious agents associated with shearling bedpads. I. Effect of laundering with detergent-disinfectant combinations of polio and vaccinia viruses. Appl. Microbiol. **19**:53-59.
21. Snedecor, G. W., and W. C. Cochran. 1967. Statistical methods, 6th ed. Iowa State University Press, Ames.
22. Songer, J. R., J. F. Sullivan, and J. W. Hurd. 1963. Testing air-filtering systems. I. Procedure for testing high efficiency air filters on exhaust systems. Appl. Microbiol. **11**:394-397.
23. Spillard, M. A. 1964. Laundering can break the infection chain—or be just another link. Mod. Hosp. **103**(4):102-107.
24. Straka, R. P., and J. L. Stokes. 1957. Rapid destruction of bacteria in commonly used diluents and its elimination. Appl. Microbiol. **5**:21-25.
25. Thomas, J. C., and M. van den Ende. 1941. The reduction of dust-borne bacteria in the air of hospital wards by liquid paraffin treatment of bedclothes. Brit. Med. J. **1**:953-958.