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Bacterial Survival in Laundered Fabrics

WILLIAM G. WALTER* AND JOHN E. SCHILLINGER
Department of Microbiology, Montana State University, Bozeman, Montana 59715

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Bacterial survival was determined in linens (i) inoculated with *Staphylococcus aureus* (ii), taken from hospital isolation patients' beds, and (iii) used by students in their homes. Two different washers using temperatures of 38, 49, 54 and 60 C, respectively, for different times were employed along with a commercial tumbler dryer. Findings, after macerating the linens in a Waring blender and enumerating on nonselective media, indicate that acceptable levels of survivors can be achieved in motel and hotel linens by an 8- to 10-min wash cycle at 54 C followed by adequate drying. However, it is recommended that a wash cycle with 60 C for 10 to 13 min be employed for linens in health care factilities. The microbial significance of various laundering practices is discussed.

Permanent press or no-iron linens have become commonplace in recent years and are now used almost exclusively in motels, hotels, and rest homes. Lower wash temperatures are also becoming more common in such establishments because of inadequate water heating facilities, heating costs, and the energy shortage.

Several investigators have studied bacterial survival in laundered fabrics (2-4, 6, 7, 9-12). Although many different detergents, wash cycles, and commercial and home type machines have been used, the general consensus is that appreciable numbers of survivors increase with lower water temperatures. It has also been shown that substantial transfer of organisms from soiled linen to clean swatches of linen included in the wash load does occur (3, 11).

Public health officials recognize the potential hazards of low-temperature laundering, but find few definitive statements in the literature regarding minimum sanitary procedures. This investigation was prompted primarily by the desire of the Montana Department of Health and Environmental Sciences to promulgate regulations for laundry operations in public establishments. Our efforts have, therefore, been directed toward determining the survival of inoculated and natural populations of bacteria with different machines, wash cycles, detergents, temperatures, and exposure times.

MATERIALS AND METHODS

Equipment. A 35-pound capacity washer-extractor (model C6M, Pellerin Milnor Corp., Kenner, La.) was used with an electronic injection system for dispensing the liquid detergent, bleach, and sour (Centramatic 52, Economics Laboratory, Inc., St. Paul, Minn.). A program chart (cycle 1) was cut for automatic operation and involved the following steps: flush (2 min, high water level); detergent, 0.25% (7 min, low water level); long rinse designed for including bleach when desired (7 min, low water level); two short rinses (1 min each, high water level); and sour (3 min, low water level). A shorter program (cycle 2) included: detergent, 0.13% (10 min, low water level); one rinse (3 min, high water level); and sour (3 min, high water level). The time of heat exposure, in the detergent and long rinse steps, was varied by manually advancing the program chart. A high-alkaline, nonionic liquid detergent system was used in cycle 1 and a low-sudsing, nonionic powder detergent was added manually in cycle 2. At the given concentrations, these gave pH values of 11.0 to 11.3 and 10.0 to 10.3, respectively. The number of steps and detergent types and concentrations were set up with the Milnor machine in this manner to compare a commercial-type cycle (cycle 1) and a home-type cycle (cycle 2). A liquid sour comprised of organic acids was used in both cycles to give a final pH of 6.5 to 7.5. The detergent and long rinse step temperatures were controlled automatically by a thermostat which regulated a steam injection system. Water temperatures in the wash wheel were read directly from the machine thermometer, which was checked by a standard thermometer for accuracy.

A laundromat Maytag washer (model A17CT) set for no-iron fabrics was also employed in certain experiments. The powder detergent (0.13%) was added before the start of the wash cycle and resulted in a pH of 9.8 to 10.3. Wash water temperatures were obtained by varying the amount of hot and cold water coming into the machine. Temperatures were measured by immersing a Taylor mercury thermometer in the wash water.

In comparing the capacities of the two washing machines, the following linen weight-machine volume ratios were determined for the Milnor and the Maytag, respectively: 0.06 kg/m^3 and 0.11 kg/m^3. These loading ratios were based on 13.6 kg of linen used for a load for both cycles in the Milnor machine and 4.2 kg

loaded into the Maytag washer. Water volumes of approximately 52 liters at low level and 66 liters at the high level setting for the Milnor, and approximately 27 liters for the Maytag, resulted in linen weight-water volume ratios of 0.21, 0.27, and 0.14 kg/l, respectively.

Drying of the linens (12 sheets/load) was accomplished in a 50-lb capacity Huebsch gas dryer (model 37 BG, Huebsch Originators, Milwaukee, Wisc.) for approximately 16 min at the medium temperature setting, followed by a 4 min cool-down period. This gave a finished product of proper moisture content for folding and storing without wrinkles.

Organisms for inoculation. Cultures of *Staphyloccus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352 were prepared by transferring a loopful of cells from a 24-h slant to a 250-ml flask containing 50 ml of tryptic soy broth (Difco) and incubating at 35 C for 24 h. The broth cultures were mixed on a magnetic stirrer for 5 min, and 0.1-ml portions were pipetted onto sterile 6.5-cm^2 swatches cut from 50% cotton-50% polyester sheeting. To provide a convenient test material more representative of field conditions, the inoculated swatches were dried at room temperature for 4 h on a sterilized screen in a covered pan. The dried swatches were then aseptically inserted into 10 pockets (approximately 5 by 5 cm) sewn to one side of a colored no-iron pillowcase. The colored pillowcase permitted easy identification in selecting samples from a full load in the washer or dryer. Ten of the swatches were immediately macerated in a sterile Waring blender and serially diluted with phosphate buffer (1). Counts from these swatches (as $\log_{10}$) usually ranged between 6.2 and 7.2 bacteria/cm^2.

Sampling. Effluent samples were obtained in sterile 250-ml flasks from a valve located on the underside of the washer drain pipe. They were collected after the various wash steps and portions were plated with tryptic soy agar plus 0.5% yeast extract. Total alkalinity measurements, expressed as milligrams of $CaCO_3$ per liter, were performed according to *Standard Methods* (1) and ranged between 500 to 600 in the detergent step of cycle 1, 600 to 700 in cycle 2, and 400 to 500 in the Maytag detergent step. A Chemtrix pH meter type 40E (Chemtrix Inc. Hillsboro, Ore.) was used for all effluent pH determinations. When chlorine bleach was added in certain runs, the residual chlorine in the effluent was determined by the iodometric technique (1).

Sterilized pillowcases each containing 10 inoculated swatches were aseptically placed in the washer and washed with a load of clean no-iron sheets that had previously been rinsed in the Milnor machine at 80 C for 15 min to destroy most bacteria that may have been present. Eight pillowcases were washed in each load, with four being removed after the wash cycle and four after the dry cycle. The swatches were then aseptically removed from the pockets with forceps and placed in sterile Waring blenders. Fifty milliliters of sterile 0.2% Tween 80 in phosphate buffer with 0.04% $Na_2S_2O_3$ were transferred from premeasured blanks to the blender cups. The 10 swatches were macerated for 2 min at low speed, and the diluent was allowed to stand for 1 to 2 min to allow foam to subside. Serial dilutions were plated with tryptic soy agar plus yeast extract. The plates were incubated for 48 h at 35 C and counted with the aid of an electronic colony counter (New Brunswick Scientific Co., New Brunswick, N.J.).

On four different occasions, 10 university students were furnished clean no-iron sheets and pillowcases for home use. After 1 week the linens were returned to the laboratory and 10 swatches were cut randomly with alcohol-flamed scissors from each pillowcase for testing. The linens were washed in the Milnor machine (cycle 2) and dried as usual. Subsequently, 10 other swatches were cut and tested.

Soiled hospital linen (cotton sheets and pillowcases) was obtained on eight occasions from the isolation ward of the Bozeman Deaconess Hospital. These linens usually came from patients with a staphylococcal infection. Ten swatches were cut from different locations on each piece of linen before washing in the Milnor machine (cycle 2) and again after the wash and drying cycles. The swatches were macerated and plated in the usual manner, and selected colonies were picked for coagulase testing. Twenty-four-hour cultures grown on tryptic soy agar plus yeast extract slants were tested, and only those giving a 4+ reaction in the tube test (Difco) were considered positive.

A Sigma 7 computer was employed to statistically examine the bacterial counts ($\log_{10}$) with analysis of variance and the Newman-Keuls test for a multiple comparison of means (8). Log_{10} counts were used because it was felt that the log transformation would give a better approximation of a normal distribution. Differences designated as significant were all judged at the 0.05 level of probability.

RESULTS AND DISCUSSION

The relative resistance of *S. aureus* and *K. pneumoniae* to environmental stress was tested by washing and drying swatches inoculated with the organisms. Table 1 shows the numbers (expressed as $\log_{10}$) of organisms surviving after washing in the Milnor machine (cycle 1) with 13 min of heat exposure and after drying. The gram-positive *S. aureus* was generally more

TABLE 1. *Geometric means ($\log_{10}$) of S. aureus and K. pneumoniae per square centimeter after washing and drying*[a]

Cycle	Wash temp (C)	*S. aureus*	*K. pneumoniae*
Before washing		7.17	5.84
After washing	38	3.72	0.59
	49	0.42	0.56
After drying	38	1.36	0.00
	49	0.27	0.00

[a] Wash cycle 1 with heat exposure time of 13 min.

resistant to washing and drying than the gram-negative *K. pneumoniae*; hence the former was employed exclusively in subsequent inoculation experiments.

Effluent counts. Numbers of *S. aureus* ($\log_{10}$) in the effluent samples ranged from 2.00 to 3.70/ml in the rinse steps with a wash temperature of 38 C, 1.08 to 2.60/ml at 49 C, and 0.48 to 2.48/ml at 60 C. The final rinse (sour) had very low numbers of organisms (0 to 1.30/ml), regardless of the wash water temperatures employed. However, the effluent counts seemed to bear no relationship to the numbers of organisms surviving in the linen itself so the data are not included in subsequent tables.

Inoculation trials. The results from 19 different batches of linens inoculated with *S. aureus* and washed in the Milnor machine (cycle 1) at different times and temperatures, and subsequently dried for about 16 min, are presented in Table 2. It is evident that as wash times and temperatures increase there is a significant decrease in numbers of *S. aureus*. Practically no organisms survived the drying treatment when linens had been washed at 49 C or above for 8 min.

In other experiments the wash temperatures of 38, 49, 54, and 60 C were maintained for 10 min in the Milnor machine (cycle 2) and in the Maytag set for the perma-press cycle. The numbers of inoculated *S. aureus* ($\log_{10}$) surviving after washing and drying are shown in Fig. 1. The points on the curves represent 21 different batches of laundry run on various occasions over a period of several months. This time lapse could result in some variations of the inoculum, washing and drying temperatures, and the ambient humidity. However, the data indicate that when using the shorter cycle 2 with the Milnor washer and the Maytag with the perma-press setting, a wash temperature of approximately 54 C is necessary to cause significant reductions in survivors after the completed treatment. Regression analysis of data (5-, 8-, and 13-min runs) from the longer treatments with the Milnor (cycle 1) was used to give an approximate 10-min count. This indicated no *S. aureus* survivors at the end of the drying cycle when a wash temperature of about 49 C was employed. This information was plotted in Fig. 1 for comparative purposes. The results of the three cycles were statistically different at 49 C but not at the 54 C treatment. The fact that the Maytag counts were lower than the Milnor cycle 2 counts suggests that the lower linen-water volume ratio in the Maytag may largely account for the increased bacterial reduction.

TABLE 2. *Survival of S. aureus in linens after washing at different exposure times and temperatures, (cycle 1) and after drying*[a]

Wash heat exposure time (min)	Wash temp (C)	Initial count	After washing	After drying
5	38	7.00[b]	6.76[c]	3.87[c]
	49	6.89	3.09(a)[d]	1.56(a)
	54	7.34	0.89(b)	0.10(b)
	60	6.18	0.00(c)	0.00(b)
8	38	7.00[b]	4.35[c]	2.17[c]
	49	7.04	1.12(a)	0.00(a)
	54	6.97	0.74(b)	0.00(a)
	60	7.69	0.16(c)	0.09(a)
13	38	7.11	4.26(a)	1.76(a)
	49	7.45	0.77(b)	0.12(b)
	54	6.34	0.34(bc)	0.00(b)
	60	7.08	0.00(c)	0.00(b)

[a] Estimates for 38 C counts at 5 and 8 min were made with regression analysis of data at the other temperatures.

[b] Estimate based on average of other initial runs.

[c] Estimate based on regression analysis.

[d] Letters in parentheses indicate statistical differences among means in respective groups.

Student-used linens. The sheets and pillowcases used by the students in their residences for 1 week were laundered in the Milnor machine (cycle 2) with a wash heat time of 10 min and the usual drying period. The numbers of organisms ($\log_{10}$) found on pillowcases before and after laundering at 38, 43, 49, and 54 C are given in Table 3. The geometric means of organisms initially present on 10 pillowcases in

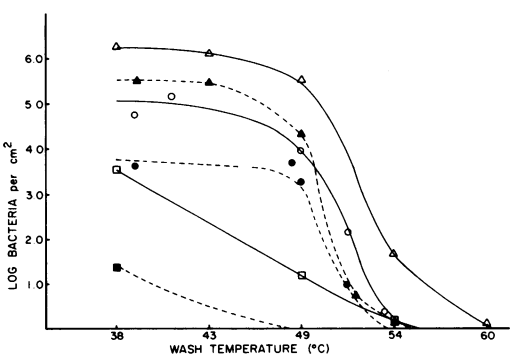

FIG. 1. *Effects of different wash cycles, temperatures (with 10-min heat exposures), and drying on survival of S. aureus. Symbols:* ○, *Maytag no-iron washed;* □, *Milnor cycle 1 washed;* △, *Milnor cycle 2 washed;* ●, *Maytag no-iron dried;* ■, *Milnor cycle 1 dried; and* ▲, *Milnor cycle 2 dried. Cycle 1 data points were estimated from regression analysis of cycle 1 data at other time exposures.*

each trial were about 2.7 cm^2 or roughly 4 to 6 logs lower than the numbers of *S. aureus* employed in the inoculation experiments. However, the numbers surviving the laundering process were somewhat higher, possibly indicating a hardier flora occurring in nature. Wash temperatures of 38, 43, 49, and 54 C resulted in a decrease of survivors after laundering of 34, 54, 68 and 94%, respectively. Statistical analyses of survivors showed significant differences among the means of each temperature trial. Gram stains from selected colonies were made. Of 38 stains from survivors of the 38 C trial, 37% were gram-positive rods and 63% were gram-positive cocci. Out of 28 survivors at 49 C, 72% were gram-positive rods, 14% were gram-positive cocci, and 14% were molds. Further identification was not undertaken.

Hospital linens. Bacterial densities in hospital isolation patient linen determined before and after washing with the Milnor machine (cycle 2) and drying are shown in Table 4. Initial counts varied greatly because of large differences in the degree and type of soiling from different patients and from variations in times the linen had remained on the beds. In one instance (Table 4, run 4) the sheet was extensively soiled from a draining leg wound and the extent of contamination was about 10,000 organisms/cm^2. However, after the wash cycle at 43 C for 10 min and drying, the count was $<10/cm^2$ although some of the survivors were coagulase-positive, gram-positive cocci. From this experiment it is obvious that laundering at 43 C was not adequate to insure a pathogen-free sheet. Similar results were obtained in run 2. However, in runs 1, 3, and 5 no survivors were found. Other investigators (4, 5, 11) agree that a potential hazard exists at such low laundering temperatures. Wiksell et al. (11) reported large numbers of *S. aureus* surviving on sheeting after washing at 24, 35, and 46 C, respectively. The presence of even low numbers of bacteria on some of the linens point to a potential health hazard in hospital isolation materials washed at 38 or 43 C. This fact was further accentuated when limited diagnostic tests were made. Gram stains of 72 colonies surviving runs 1 and 2 showed 51% gram-positive cocci and 46% gram-positive rods. Survivors of runs 3 and 4 showed 79% of 64 colonies to be gram-positive cocci, 10% gram-positive rods, and 11% molds. Twenty-five colonies were picked at random from each set of plates from runs 2 and 4. One organism from run 2 and 10 organisms from run 4 were coagulase-positive gram-positive cocci and appeared morphologically to be typical staphylococci, thus indicating the probable survival of pathogens on the contaminated linens.

Effects of chlorine. Some state regulations (New Hamphsire and North Dakota) specify that a suitable chemical disinfectant be added at some step of the wash cycle. Several runs were made to note the effect of adding chlorine to the Milnor machine (cycle 1) when *S. aureus*-inoculated swatches in pillowcases were included with a regular load of clean sheets. The results (Table 5) clearly indicate the value of increased concentrations of chlorine when employing a low wash temperature of 38 C. Granted that some colored materials should not be treated with a bleach, no proven and accepted chemical substitutes are commercially available at this time. In situations where serious contamination occurs, it may be advisable to use high temperatures or bleach which may reduce the useful life of the fabric but

TABLE 3. *Geometric means (log_{10}) of bacteria per square centimeter on student-used linen before and after laundering (cycle 2)*

Cycle	Run 1	Run 2	Run 3	Run 4
Temp (C)	38	43	49	54
Before laundering ..	2.47[a](a)	2.68(a)	2.67(a)	2.72(a)
After laundering ...	1.64(a)	1.23(b)	0.85(c)	0.17(d)

[a] Letters in parentheses reading across denote statistical differences among means at 0.05 level of probability.

TABLE 4. *Numbers of bacteria (log_{10}) per square centimeter from soiled hospital isolation patient linen before and after laundering (cycle 2)*

Cycle	Run 1	Run 2	Run 3	Run 4	Run 5
Wash temp (C)	38	38	43	43	49
Before laundering					
Range	1.53 to 2.59	1.45 to 3.36	2.36 to 3.36	2.83 to 4.97	0.60 to 1.28
Geometric mean	1.85	2.46	2.88	3.98	0.78
After laundering					
Range	0.0 to 0.0	0.95 to 1.99	0.0 to 0.0	0.0 to 1.81	0.0 to 0.0
Geometric mean	0.0	1.36	0.0	0.60	0.0

TABLE 5. *Numbers of S. aureus (log_{10}) surviving the laundering process with different chlorine concentrations and temperatures*[a]

Cycle	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Temp (C)	38	38	38	38	49	49
Cl_2 (mg/liter)	0	69	131	213	0	82
Initial count	7.11	7.36	7.34	7.18	7.45	7.76
After washing	4.26	1.08	0.25	.04	0.77	0.67
After drying	1.76	0.00	0.07	0.00	0.12	0.00

[a] Milnor machine with cycle 1 and 13-min heat exposure. Bleach was added in third step.

which will insure the safety of the materials. This important factor should be considered prior to purchasing such fabrics.

There is a dearth of specific state regulations relating to the laundering of linens in hotels, motels, nursing homes, and hospitals. Information gathered from 38 states indicated that 27 of these had no laws, several were considering some stipulations, and 10 states had some very general regulations. Of the latter, four specify washing at 74 C for 25 min, one requires washing at 71 C for 30 min, another at 65 C for 20 min, and three others suggest temperatures of 60 to 82 C without indicating times.

On the basis of our studies it is clear that it is neither feasible nor practical for a regulating agency to routinely perform laboratory tests on linens to ascertain whether they have been laundered properly. Such testing is time consuming and destructive to the linen. McNeil (3) isolated many species of bacteria from swatches attached to linen but not from the rinse water, so redeposition appeared to her "to be a realistic means of evaluating effectiveness." Our own experiments with several motel laundry runs showed bacteria deposited on sterile swatches to be of the same magnitude as the numbers obtained from sampling the actual linens. Further research along these lines might establish a definite relationship, but the techniques are still as detailed and cumbersome as actual linen sampling.

With increased interest in conserving energy and the widespread advertising campaigns continually bombarding the public to employ lower temperatures for washing, it behooves public health workers to analyze research findings in developing pragmatic regulations that can be monitored and enforced. To our knowledge, no official guidelines or standards for numbers of bacteria in finished laundered linens have ever been proposed. It is likely that such numbers would have little practical significance based on the difficulties of obtaining such counts alluded to previously. However, it does seem worthwhile and imperative to recommend some times and temperatures which, based on these investigations, can achieve levels of microbes of $0.2/cm^2$ in properly laundered and stored linens. This number is the lower limit of organisms detectable by our methods and appears to be equivalent to complete pathogen removal.

It appears that linens from hotels and motels that generally are changed daily could be processed adequately at a wash temperature of 54 C for 8 to 10 min and subsequently dried in a hot air tumbler dryer at a temperature high enough to expedite the process. Equally satisfactory results could probably be obtained with a temperature of 60 C and a wash heat exposure time of 5 min. For linens employed in health care facilities and used by a great diversity of patients, a wash temperature of 60 C for 10 to 13 min followed by drying is recommended. The addition of bleach gives an added degree of safety in all cases.

It must be stressed that even the above laundering procedures will not provide safe linens to the people who use them unless conscientious efforts at good housekeeping and sanitation are followed throughout the entire laundry procedure. Practices which eliminate human error and forgetfulness, such as the use of programmed washers and controlled dispensing of chemicals, are recommended. Physical separation of soiled and clean linen areas, separate soiled and clean linen handlers and carts, and care in transporting and storing linen should eliminate almost all linen recontamination and insure bacteriological safety.

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