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Impact of Detergent Systems on Bacterial Survival on Laundered Fabrics

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The survival of *Staphylococcus aureus* was determined from inoculated swatches laundered in either a phosphate or a phosphate-substitute detergent. In a Plackett-Burman design study, the independent variables of detergent type, concentration, and variation, wash water temperature, soil load, cycle time, and water hardness were assigned high and low values. Wash water temperatures of 27, 38, 49, and 60°C were employed. Viable bacteria were recovered from macerated swatches. Statistical analysis disclosed that there was no practical difference in the ability of phosphate or phosphate-substitute detergents to reduce the level of *S. aureus* on the laundered swatches in this controlled design. Analysis did reveal that water temperature was the most significant independent variable. The remaining variables did not appear to have any practical significance upon bacterial reduction. This bacteriological study did not evaluate other essential detergent properties.

Considerable data have been reported on bacterial survival after the laundry process (1, 3, 5, 6, 8, 10, 12, 14-18, 20, 22-24, 27-33). Studies have evaluated fabric type (19, 30), wash temperature (18, 27), laundry design (3, 5, 14, 28), recontamination of fabrics (6, 24), and survival of viruses in laundry systems (8, 22, 23). There is general agreement that bacterial survival increases with reduced wash temperatures (14, 20, 27, 28). With the advent of energy awareness, there has been increased interest in low-temperature laundry. Thus, the survival of potential pathogens on laundered fabric has become a significant public health concern (28). This concern may be of special importance in the control of nosocomial infections. Hospital patients, having less resistance to infection than healthy people, may become infected by less virulent organisms or by lower infective doses than healthy people (12).

Detergents consist of surfactants and builders. Builders control properties in wash water that may reduce the surfactant's effectiveness. Walter and Schillinger (28) demonstrated that the use of built detergents and high wash water temperatures can significantly reduce the number of surviving organisms on laundered fabrics. With the lowering of laundry wash water temperatures, the question arises as to the importance of a well-built detergent in the reduction of microorganisms on laundered fabrics.

The contributions of phosphates to the cleaning of fabrics are well documented (9, 13, 21, 33). Detergents containing high levels of phosphates are much more effective in removing soil from fabrics than low-phosphate and nonphosphate detergents, especially in hard water and at low detergent concentrations (R. L. Glee, Ph.D. thesis, University of Wisconsin, Madison, 1973). Carfagno and Fuchs stated that studies have found that the detergency of a variety of nonphosphate detergent products and soap was consistently poorer than the detergency of typical phosphate formulations. Also, phosphate products exhibit superior cleaning performance, do not cause a buildup of deposits, and are effective in the removal of mineral residues on fabrics and washer equipment (P. P. Carfagno and R. J. Fuchs, Abstr. Annu. Spring Meet. Am. Oil Chem. Soc., abstr. no. 95, p. 285A, 1974). However, recent legislative actions restricting phosphates in on-premise laundry detergents pose further considerations to the question of whether a fabric is sanitary if it is not clean. Marmo (14) indicated that soil removal and bacterial reduction are linked. Wiksell et al. (30) stated that although detergents might aid in the physical removal of microorganisms, the detergent type is not a significant factor in bacterial reduction.

Reported investigations have not combined the factors of water hardness, soil load, wash water temperature, cycle time, and detergent type, concentration, and variation into a single study designed for the purpose of determining the significance of each variable in reducing bacteria on laundered fabric. The primary objective of this study was to determine whether there is a significant difference between phosphate and phosphate-substitute detergents in the reduction of *Staphylococcus aureus* from

laundered fabric swatches in a controlled experimental design.

MATERIALS AND METHODS

Experimental design. This investigation involved two 16-experiment Plackett-Burman test designs (25) and eight control washings without detergent. In this highly fractionated factorial design system, high (+) and low (−) values were assigned for each independent variable. The order for testing was determined randomly. An in-cycle inhibition study evaluated the relative inhibitory effects of the detergents upon the test organisms.

Variables. The design variables comprised the following: detergent type (phosphate or phosphate-substitute), detergent concentration, detergent variation, water hardness, temperature, and soil load. Both detergents were formulated identically in terms of optical brightener, nonionic surfactant, antiredeposition agent, buffering agent, and alkalinity source. In addition, the phosphate detergents contained either 25.00% (high detergent variation) or 12.50% (low detergent variation) sodium tripolyphosphate, whereas the phosphate-substitute formulation contained either 4.00% (high) or 2.00% (low) polyelectrolyte as sequestrants and water-conditioning agents. An inactive filler completed each formulation. The pH of the detergent solutions was 10.0.

Wash water temperatures were paired at 60 and 38°C and at 49 and 27°C. Water conditions were 240 ppm (240 mg/liter) (high) and less than 1 ppm (low) (hardness in parts per million as Ca^{2+} and Mg^{2+}). The detergent was added manually at either a 0.15% (85 g) or a 0.30% (170 g) concentration. The soil, 17 g of Bandy Black Research Clay (H. C. Spinks Clay Co., Paris, Tenn.) plus 19 ml of corn oil, was added manually at the detergent spout to provide a 300-ppm soil load. The soil load was either absent (low) or present (high).

Equipment. An 11.32-kg (25-lb) capacity washer-extractor (WASHMASTER [Economics Laboratory, Inc., St. Paul, Minn.] 25 LST, Econ Laundry Systems, Memphis, Tenn.) was used for the entire test period. The automated system provided a consistent operation which used 182 to 197 liters of water per cycle. A program chart was cut for automatic operation. Cycle 1 combined the following steps (and their respective times): fill, 35 s; wash, 13 min; drain, 53 s; fill, 34 s; rinse, 2 min and 34 s; drain, 1 min and 21 s; fill, 20 s; rinse, 2 min and 43 s; drain, 52 s; and extract, 2 min and 52 s. Cycle 2 reduced the wash step to 5 min. This was accomplished by manually advancing the program chart. All other steps remained the same. The powdered detergent was added manually before the start of the cycle.

Before the test cycle, the washer-extractor was prepared in the following manner. The WASHMASTER supply hoses were connected to the appropriate water line for the required hardness level. The water temperature was adjusted by varying the amount of hot and cold water entering the machine. This was measured with a standard bayonet thermometer at the water inlet elbow. To sanitize tub components, a sodium hypochlorite solution yielding 100 ppm of available chlorine (75 ml of XY-12 Liquid Sanitizer, Klenzade Div., Economics Laboratory, Inc.) was added to the machine at the detergent spout. The washer-extractor was then allowed to run through cycle 1.

Organism. *S. aureus* was chosen for its relative resistance to the laundry process (28) and because it is of special public health and nosocomial concern (7, 11). *S. aureus* ATCC 6538 was the test organism for the study.

Bacterial inoculation. A culture of *S. aureus* was prepared by transferring 1.0 ml of a 24-h culture tube containing 10 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) to a 300-ml flask (no. 2574, Bellco Glass, Inc., Vineland, N.J.) containing 50 ml of Trypticase soy broth. The culture flask was incubated for 24 h at 35°C. The incubated broth culture was mixed for 2 min by gentle swirling of the flask. Samples of 0.1 ml of the flask culture were pipetted onto sterile 6.5-cm^2 fabric swatches cut from 50% cotton–50% polyester sheeting material. Ten swatches (five swatches to be washed and five swatches as the unwashed control) were aseptically placed in two separate sterile petri dishes for 1 h at 24°C in a vacuum desiccator (Pyrex Brand, Corning Glass Works, Corning, N.Y.). With sterile forceps, five dried swatches were aseptically inserted into separate pockets (approximately 5 cm^2) sewn to one side of a sterile colored cloth napkin. The remaining five dried swatches were individually macerated in separate Waring blender jars (model 5011, Waring Products Div., New Hartford, Conn.) containing 99 ml of sterile phosphate-buffered water (2) with 0.2% Tween 80. After the foam had settled (approximately 1 min), serial dilutions were plated (2) in duplicate with Trypticase soy agar plus 0.01% sodium pyruvate. The plates were incubated for 48 h at 35°C and counted with an electronic colony counter (New Brunswick Scientific Co., New Brunswick, N.J.). Counts were recorded as colony-forming units per centimeter squared.

After the sanitization of the washer-extractor, the napkin containing the five inoculated swatches and 6.8 kg of sterilized bed sheets as filler were aseptically placed in the washer-extractor. The appropriate detergent and designated soil load as shown in Tables 1 and 2 were added to the wash load, and the cycle was activated. At the conclusion of the cycle, the napkin was aseptically removed, and the five swatches were transferred with sterile forceps to separate Waring blender jars for maceration. Serial dilutions were plated in duplicate in the same manner as the unwashed control swatches.

Statistical analysis. The reduction of viable bacteria after the laundry process was expressed as the mean negative logarithmic reduction (MNLR). This expression was calculated by dividing the resulting washed count by the unwashed count and finding the negative logarithm of the resulting number. The statistical analysis of the MNLR data was a modified Student *t* test (25). Apparent effects of each variable were calculated as the average MNLR difference between the high (+) and low (−) levels of the variable. The pseudovariables provided an estimate of the standard error of the experiment. With the *t* test, the apparent effect of a variable was compared with the standard error to determine the statistical significance, if any, of the variable.

TABLE 1. *Bacterial reductions on fabric laundered with phosphate detergent*

Wash cycle time (min)	Water temp(°C)	Soil load	Detergent variation (%)	Detergent concn (%)	Water hardness (ppm)	Unwashed count (CFU/cm²)[a]	Washed count (CFU/cm²)[a]	MNLR
13	60	+	12.5	0.15	240	3.2×10^7	<100	>5.505
13	60	−	25.0	0.15	<1	2.6×10^7	<100	>5.415
5	60	−	12.5	0.30	240	9.6×10^6	<100	>4.982
5	60	+	25.0	0.30	<1	9.0×10^6	<100	>4.954
13	49	−	25.0	0.30	240	2.9×10^7	1×10^2	5.462
13	38	−	25.0	0.30	240	1.1×10^7	1.1×10^2	5.000
13	38	+	12.5	0.30	<1	1.8×10^6	1.9×10^1	4.977
5	49	+	25.0	0.15	240	1.4×10^7	1.5×10^2	4.970
5	38	+	25.0	0.15	240	3.0×10^7	8.5×10^2	4.548
5	49	−	12.5	0.15	<1	7.8×10^6	6.1×10^2	4.107
13	49	+	12.5	0.30	<1	1.9×10^6	3.7×10^2	3.711
5	38	−	12.5	0.15	<1	4.0×10^7	1.2×10^5	2.523
5	27	−	12.5	0.30	240	3.2×10^7	2.9×10^6	1.043
13	27	−	25.0	0.15	<1	5.0×10^7	5.4×10^6	0.967
5	27	+	25.0	0.30	<1	3.4×10^7	5.6×10^6	0.783
13	27	+	12.5	0.15	240	1.2×10^7	3.7×10^6	0.511

[a] Mean of duplicate samples for five swatches. CFU, Colony-forming units.

TABLE 2. *Bacterial reductions on fabric laundered with phosphate-substitute detergent*

Wash cycle time (min)	Water temp (°C)	Soil load	Detergent variation (%)	Detergent concn (%)	Water hardness (ppm)	Unwashed count (CFU/cm²)[a]	Washed count (CFU/cm²)[a]	MNLR
5	60	−	2.0	0.30	240	2.4×10^7	<100	>5.380
13	60	−	4.0	0.15	<1	7.0×10^6	<100	>4.845
5	60	+	4.0	0.30	<1	7.2×10^5	<100	>3.857
13	60	+	2.0	0.15	240	3.4×10^5	<100	>3.532
13	49	+	2.0	0.30	<1	4.3×10^7	1.3×10^1	6.520
13	49	−	4.0	0.30	240	8.6×10^6	1.0×10^1	5.935
5	49	+	4.0	0.15	240	9.1×10^6	2.7×10^1	5.528
13	38	−	4.0	0.30	240	8.5×10^6	1.6×10^2	4.725
5	38	+	4.0	0.15	240	3.8×10^7	3.7×10^3	4.012
13	38	+	2.0	0.30	<1	4.0×10^7	5.1×10^3	3.895
5	49	−	2.0	0.15	<1	5.4×10^7	6.2×10^4	2.940
5	27	−	2.0	0.30	240	4.2×10^6	9.4×10^5	0.650
5	38	−	2.0	0.15	<1	1.1×10^6	1.2×10^5	0.962
5	27	+	4.0	0.30	<1	5.0×10^7	5.4×10^6	0.966
13	27	+	2.0	0.15	240	2.9×10^7	6.4×10^6	0.656
13	27	−	4.0	0.15	<1	2.6×10^7	8.8×10^6	0.471

[a] Mean of duplicate samples for five swatches. CFU, Colony-forming units.

In cycle. Solutions (100 ml each) were prepared of the following: 0.3% phosphate detergent, 0.3% phosphate-substitute detergent, phosphate-buffered water, and Trypticase soy broth. A 0.1-ml sample of a 24-h *S. aureus* broth culture was added to each solution, and the solutions were held at 35°C. Samples (1.0 ml each) of the inoculated solution were plated for recovery of viable organisms at 0, 2, 4, 6, 24, and 48 h on Trypticase soy agar.

RESULTS AND DISCUSSION

Reductions of inoculated *S. aureus* populations from fabric swatches subject to independent variables in a Plackett-Burman design and laundered in phosphate, phosphate-substitute, or the absence of detergent are shown in Tables 1, 2, and 3. The average MNLR were 3.716 for the phosphate detergent design, 3.430 for the phosphate-substitute detergent design, and 3.554 for the detergent-free design. Within the test parameters, an analysis of the average MNLRs revealed that differences between detergent types were not statistically significant in bacterial reduction. Both detergent types, although determined not to be a direct factor in microbial reductions, aided in the physical removal of microorganisms by lowering the surface tension (23). These observations agree with those reported by Sidwell et al. (23) and Wiksell et al. (30), although those studies compared detergent types in terms of surfactant variation (anionic and nonionic), whereas this study compared detergent types in terms of sequestration and antiredeposition agents (sodium tripolyphosphate and polyelectrolyte).

TABLE 3. *Bacterial reductions on fabric laundered in the absence of detergent*

Wash cycle time (min)	Water temp (°C)	Soil load	Detergent variation (%)	Detergent concn (%)	Water hardness (ppm)	Unwashed count (CFU/cm²)[a]	Washed count (CFU/cm²)[a]	MNLR
5	60	−	0.0	0.0	240	1.8×10^8	>100	>6.255
13	60	+	0.0	0.0	240	1.2×10^8	>100	>6.079
5	49	−	0.0	0.0	>1	3.6×10^8	6.0×10^2	5.778
13	49	+	0.0	0.0	>1	3.6×10^8	6.4×10^2	5.750
13	38	+	0.0	0.0	>1	7.0×10^6	7.9×10^4	1.947
5	38	−	0.0	0.0	>1	8.0×10^6	1.1×10^5	1.862
5	27	−	0.0	0.0	240	4.3×10^8	1.7×10^8	0.403
13	27	+	0.0	0.0	240	4.3×10^8	1.9×10^8	0.355

[a] Mean of duplicate samples for five swatches. CFU, Colony-forming units.

A statistical analysis of the data indicated that water temperature is the primary factor in reducing viable *S. aureus* cells recovered from fabric. In the phosphate detergent experiments, water temperature was statistically significant at the 98% confidence level; in the phosphate-substitute detergent experiments, it was statistically significant at the 90% confidence level. The other independent variables (i.e., soil load, water hardness, detergent formulation variation, and detergent concentration) demonstrated irresolute effect on the resulting microbial densities at the 80% confidence level for the swatches laundered in the phosphate detergent and no measurable effect in the phosphate-substitute detergent design. It can be noted from the data that the influence of these independent variables seemed to increase as the water temperature was reduced from 38 to 27°C. This is exemplified in part by the greater than 3-log difference recorded at the two temperatures, but because of the predominant effect of water temperature, statistical significance values for each of the remaining independent variables could not be assigned.

Averages of the computed MNLRs for the four trials at each water temperature laundered in either detergent type are shown in Table 4. Average MNLRs of 0.83 for phosphate detergent trials, 0.77 for phosphate-substitute trials, and 0.38 for detergent-free trials were calculated from swatches laundered in water at 27°C. Water temperatures of 38 and 49°C significantly reduced the number of recoverable cells from the washed swatches by an average of 4.26 and 4.56 logs in the phosphate detergent trials, 3.40 and 5.21 logs in the phosphate-substitute detergent trials, and 1.91 and 5.76 logs in the detergent-free trials for each of the respective temperatures. Water temperature trials at 60°C reduced the microbial counts to below the analytical limit of detectability and therefore created the "greater than" expression of the data. These reductions were attributed to the washing process and dilution factor. Studies by Eisenberg (9) showed that soil removal characteristics of detergent solutions improved as water temperatures were increased up to 71°C. Other studies (14, 20, 27, 28) stated the importance of water temperature in reducing bacteria on laundered fabrics. The data from this study also demonstrate the importance of water temperature in the laundry process.

TABLE 4. *Comparative average MNLRs of phosphate detergent, phosphate-substitute detergent, and detergent-free cycles at the tested water temperatures*

Temp (°C)	Avg MNLR		
	Phosphate detergent	Phosphate-substitute detergent	Detergent-free
27	0.83	0.77	0.38
38	4.26	3.40	1.91
49	4.56	5.23	5.76
60	>5.21	>4.40	>6.17

The exact mode of action contributing to the observed microbial reductions is difficult to determine. This is due to the influence of such factors as water temperature, physical dilution, mechanical and chemical action, and the time during which the fabric is being processed. Marmo (14) stated that bacterial removal occurs in the same manner as soil removal. Bacteria affixed to soil or fibers of the fabric are dislodged from the fabric by mechanical and chemical action. These particles are suspended in the wash solution and rinsed away, consequently reducing the number of bacteria recovered. Study data appeared to demonstrate that this process was enhanced as water temperature increased to 60°C.

Potential bactericidal activities of nonionic phosphate and phosphate-substitute detergents are shown in Table 5. At 6 h after inoculation of the detergent solution, a 1.1-log reduction was observed for the phosphate detergent, and a 1.4-

TABLE 5. *Comparative in-cycle growth inhibition of S. aureus in 0.3% phosphate detergent (25.0% sodium tripolyphosphate) solution, 0.3% phosphate-substitute detergent (4.0% polyelectrolyte) solution, phosphate-buffered water, and Trypticase soy broth plus pyruvate*

Growth medium	Mean CFU/ml[a] at:					
	0 h	2 h	4 h	6 h	24 h	48 h
0.3% Phosphate detergent solution	2.7×10^6	6.1×10^5	4.4×10^5	1.1×10^5	100	100
0.3% Phosphate-substitute detergent solution	2.8×10^6	6.0×10^5	1.8×10^5	6.4×10^4	100	100
Phosphate-buffered water	2.8×10^6	2.9×10^6	2.7×10^6	2.5×10^6	2.3×10^6	1.3×10^6
Trypticase soy broth plus pyruvate	3.0×10^6	3.3×10^6	1.7×10^7	2.5×10^8	3.3×10^9	1.9×10^9

[a] Mean of five duplicative trials. CFU, Colony-forming units.

log reduction was observed for phosphate-substitute detergent. After 24 h, the inoculated population was reduced to less than the analytical limit of detectability. In contrast, the buffered-water control-inoculated population did not change significantly, whereas the Trypticase soy broth-inoculated population significantly increased (3 logs). The *S. aureus* reductions in the inoculated detergent solutions were not attributed to bactericidal properties of the nonionic detergents, but rather to extended exposure to the alkaline environment of the solution (pH 10.0) (26). A growth pH range of 4.3 to 9.3 has been reported for *S. aureus* (4). The alkalinity of the laundering solution was of no practical bactericidal significance because the wash step was not longer than 13 min. In addition, the pH was relatively similar in all experiments, indicating an insignificant contribution. This is similar to what was previously reported by Sidwell et al. (23).

Infection is not determined by the mere numbers present on laundered fabric. The presence of low numbers of microorganisms on laundered fabrics could serve as a potential hazard to susceptible hosts. As reported by Greene (12), actual epidemiological evidence for this transmission is not substantial. However, it is essential that the potential be recognized and that any risk be minimized. From the analysis of the data, it seems reasonable to suggest that reduced-water-temperature laundering in health care institutions has limited application because the survival of a few bacteria after laundering denotes the potential for nosocomial infections.

With the reduction or elimination of phosphate builders from laundry detergents, the potential health hazards in health care institutions utilizing phosphate-substitute detergents merit investigation. In regard to bacterial reduction, a statistical analysis of the test data could not determine a significant difference between either laundry detergent type. However, the study parameter was limited to the survival of *S. aureus* on fabrics subject to the laundry process in two detergent types, and it did not account for other significant detergent properties, such as total cleaning performance on various soil types, dispensing and solubility, effect on fabric durability, and effect on machinery service records.

Institutional laundries are responsible for providing sanitary linen. Consequently, laundry procedures employing minimum water temperatures of 49°C for hospitality industry linens and minimum water temperatures of 60°C for health care linens plus the use of built laundry detergents at manufacturer-recommended use concentrations are suggested. In addition, 10- to 13-min wash cycle times, programmed laundry machines with automatic dispensing systems, and thorough drying are also essential elements of laundry programs. It is important that all personnel be trained to employ proper procedures to help eliminate the potential transmission of pathogenic microorganisms by laundered fabric.

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