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Potentially Infectious Agents Associated with Shearling Bedpads: Effect of Laundering with Detergent-Disinfectant Combinations on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Glutaraldehyde-tanned woolskins which are used as bedpads to prevent decubitus ulcers were contaminated with *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442). Two methods of exposure, direct contact and aerosol, were used in separate experiments. Attempts were made to decrease the bacterial population placed on the woolskins by laundering them in a quaternary ammonium disinfectant, a phenolic disinfectant, or alkalinized glutaraldehyde, in combination with an anionic or nonionic detergent. The effect of a commercial detergent-sanitizer was also studied. Bacterial populations were significantly reduced in all experiments, but only laundering in glutaraldehyde in combination with either detergent resulted in maximum removal of bacteria. Viable bacteria were usually not detected in the rinse water (<1 viable organism/5 ml of rinse water).

A special glutaraldehyde-tanned shearling (woolskin) has been developed which has utility in the prevention and cure of decubitus ulcers through its use as a bedpad (3). Studies on the persistence of potential pathogenic bacteria and viruses on fabrics have shown that these microorganisms can persist for relatively long periods of time on fabrics (2, 5, 9, 10). Since the most effective and economical use of shearling bedpads by hospitals and other similar institutions will involve the laundering of these bedpads, the present study was undertaken in an attempt to develop an effective laundering procedure. This report describes the studies carried out with *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Woolskins. Shearling medical pads were obtained from A. C. Lawrence Leather Company, Peabody, Mass. They were tanned by a process based on the method developed by the U.S. Department of Agriculture, Agricultural Research Service (3).

Bacteria. The bacteria used in these studies were *S. aureus* FDA209 (ATCC 6538) and *P. aeruginosa* PRD10 (ATCC 15442) obtained from the American Type Culture Collection, Rockville, Md. The lyophilized cultures were suspended in Trypticase Soy Broth (BBL), grown for approximately 18 hr at 37 C, and then frozen in 2-ml samples and stored at about −5 C. When a culture was required for an experiment, a frozen culture was thawed, 1.0 ml was inoculated into a flask containing 100 ml of Trypticase Soy Broth, and the culture was incubated for 18 hr at 37 C with gentle agitation.

Detergents and disinfectants. The detergents and disinfectants used in this study are listed in Table 2 and have been recently described (7). A sour was used in the rinse water (106 ppm) to render a less alkaline *p*H to the laundered material (7).

Washing machine. The washing machine used was an automatic, top-loading, agitator-type (Lady Kenmore, Sears, Roebuck and Co., Chicago, Ill.). The specifications of this machine and the procedure for using it have been described (7).

Methods of exposure to bacteria. Direct contact and aerosol methods of exposure were used. For the direct contact methods, 1.0 ml of a standardized bacterial suspension (an 18-hr culture adjusted to a cell density of 1.5×10^8 cells/ml) was pipetted onto the woolskins. Exposure to aerosol was carried out in a molded plastic isolator (Germfree Laboratories, Inc., Miami, Fla.). The aerosol was produced by two De Vilbiss atomizers facing each other, and a total pressure of 13 psi of nitrogen gas was applied to these two atomizers. These methods have been described in detail elsewhere (5).

Method for determining effectiveness of wash procedures. Shearling swatches, 2 inches (5.08 cm) in

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diameter, were cut with a mechanized die. The swatches and whole shearlings were sterilized with ethylene oxide (STERI-VAC Sterilizer, 3-M Co., St. Paul, Minn.) as recently described (6). Five sterile swatches were pinned to a sterile whole shearling pad. The shearling with the attached swatches, an additional whole shearling, and five additional swatches were contaminated with bacteria by one of the exposure methods. The five additional swatches were reserved as unlaundered controls to determine the initial bacterial population density. The second shearling was used to provide balance in the washing machine and to provide additional bacteria to the total. Therefore, any reduction of the bacteria which was brought about by laundering was not a result of dilution in the wash water. The two shearlings and the five attached swatches were laundered with a test detergent and disinfectant, or detergent only, at a temperature 50 ± 6 C for 10 min. This laundering was followed by a 3-min rinse in water at a temperature of 39 ± 6 C. The sour was then added, and an additional 3-min rinse was carried out at the same temperature. A 6-min spin-dry cycle was used to remove the major portion of the water from the pad. The wash agitation speed was as follows: 70 agitations/min for 4 min, 48 agitations/min for 4 min, and then 70 agitations/min for 2 min. A 10-ml amount of the second rinse water was removed for assay of bacterial content. Immediately after the spin-dry cycle, the swatches were removed and the wool was mechanically separated from the leather (7). The wool was placed in a 40-ml homogenizer cup (Ivan Sorvall, Inc., Norwalk, Conn.). The leather was cut into small pieces and placed in another homogenizer cup. A 25-ml amount of physiological saline was added to each cup. The material was macerated by running the homogenizer, placed in an ice bath, at maximum speed for 30 sec.

Each eluate was then serially diluted tenfold from 10^0 (undiluted) to 10^{-5} with physiological saline. The number of viable bacteria was determined by plating 0.1-ml amounts of each dilution in quintuplicate on tryptic soy agar plates (15 by 100 mm, Falcon Plastic Div. of B-D Laboratories, Inc., Los Angeles, Calif.; reference 10). The plates were inverted and incubated at 37 C for 24 hr and the number of bacterial colonies were counted. The number of bacteria recovered per square inch (6.54 cm^2) of swatches was calculated (Table 1).

The least number of viable bacteria that can be detected was approximately 500 viable microorganisms/in^2 of swatch. Since 1 ml was pipetted onto a woolskin swatch and the wool and leather were harvested separately, each in 25 ml of physiological saline, this was equivalent to a 1:50 dilution. The quantity of undiluted eluate plated was 0.1 ml and, therefore, the final dilution was 1:500. Thus, if one colony in the undiluted eluate was detected, then approximately 500 bacteria must have been originally present on the swatch. In other words, if the number of viable bacteria on the swatch was less than 500 microorganisms, the method might not detect any bacteria.

The results of the effectiveness of the different laundering procedures were compared on the basis of the approximate $\log_{10}$ reduction in population (Tables 2 and 3) defined as follows: $\log_{10}$ [Bacteria per square inch of control (unlaundered) sample/bacteria per square inch of test (laundered) sample]. Since the $\log_{10}$ reduction in population depends upon the initial bacterial population density (i.e., the population density of the control sample) as well as the population density after laundering, the maximum reduction in population that could be detected under the employed experimental conditions was about 5.0 to 5.4 $\log_{10}$. For example, if the mean population density of the unlaundered (control) samples was 1.4×10^8 bacteria per square inch and if we could not detect any bacteria in the laundered samples, then the $\log_{10}$ reduction in population would be $\log_{10}(1.4 \times 10^8/5.0 \times 10^0) = >5.4$. In the experiments in which bacteria could not be recovered from the laundered swatches a > sign precedes the calculated $\log_{10}$ reduction in population value since this value indicates that at least that degree of bacterial reduction was achieved.

One milliliter of rinse water was plated in quintuplicate. Therefore, in the experiments in which bacteria were not detected in the rinse water, the cell density must be <1 viable cell/5 ml of rinse water.

Variation among test samples in a given group was usually about 1 $\log_{10}$. The samples contaminated by exposure to aerosolized bacterial cultures were randomly distributed (9). Therefore, representative sampling in the aerosol chamber was achieved by selecting at random five swatches exposed to the aerosol.

RESULTS AND DISCUSSION

The results of an experiment typical of those carried out in this study are shown in Table 1. The experiments in which *S. aureus* or *P. aeruginosa* were placed on the woolskins by direct contact or aerosol, and woolskins laundered are summarized in Tables 2 and 3.

Processing the contaminated woolskins through the washing cycles without any added detergent or disinfectant resulted in reduction of the bacterial population on the woolskins (0.5 to 2.0 $\log_{10}$). For instance, the laundering process reduced the *S. aureus* population on the wool or leather by about 2.0 $\log_{10}$ and the *P. aeruginosa* population on the wool or leather by 0.5 to 1.0 $\log_{10}$ (Tables 2 and 3).

The laundering of contaminated woolskins with a nonionic or an anionic detergent produced about the same reduction in the number of bacteria on the woolskins as that obtained by processing the woolskins through the washing procedure without any added detergent (Tables 2 and 3). In fact, washing the contaminated woolskins with the combination detergent-sanitizer did not provide any apparent advantage to the procedure (Tables 2 and 3). However, when the wool-

TABLE 1. *Effect of laundering with nonionic detergent (265 ppm) and glutaraldehyde (20,000 ppm) on the population density of Staphylococcus aureus placed on woolskins by direct contact*

Description of sample	Swatch no.	Population density[a] (organisms/in²)	Mean population density (organisms/in²)	Approx. $\log_{10}$[b] reduction in population
Bacterial control[c]—wool	1	8.4×10^7		
	2	7.1×10^7		
	3	7.2×10^7	4.6×10^7	
	4	1.1×10^6		
	5	1.3×10^6		
Test[d]—wool	6	$<5.0 \times 10^2$		
	7	$<5.0 \times 10^2$		
	8	$<5.0 \times 10^2$	$<5.0 \times 10^2$	>5.0
	9	$<5.0 \times 10^2$		
	10	$<5.0 \times 10^2$		
Bacterial control[c]—leather	11	1.1×10^6		
	12	1.1×10^6		
	13	6.8×10^5	9.0×10^5	
	14	9.8×10^5		
	15	8.2×10^5		
Test[d]—leather	16	$<5.0 \times 10^2$		
	17	$<5.0 \times 10^2$		
	18	$<5.0 \times 10^2$	$<5.0 \times 10^2$	>3.3
	19	$<5.0 \times 10^2$		
	20	$<5.0 \times 10^2$		
Rinse water		None detected[e]		

[a] One square inch = 6.45 cm².

[b] $\log_{10}$ [bacteria per square inch of control (unlaundered) sample/bacteria per square inch of test (laundered) sample].

[c] Exposed to bacteria but not processed through laundering or rinsing.

[d] Exposed to bacteria and processed through washing and rinsing as described in Materials and Methods.

[e] Cell density was <1 viable organism per 5 ml of rinse water.

skins were contaminated by exposure to an aerosol of the organisms, laundering these contaminated woolskins with the combination detergent-sanitizer did produce a 3.0 to 5.0-$\log_{10}$ reduction of the bacterial population on the wool.

In general, using a quaternary ammonium or a phenolic compound as a disinfectant produced a substantial reduction of bacteria (either *S. aureus* or *P. aeruginosa*) on the wool of the shearlings (Tables 2 and 3). By comparison these disinfectants were much less effective in reducing the bacterial population on the leather portion of the woolskins.

Glutaraldehyde was the most effective disinfectant employed in these studies. This compound has previously been reported to have bactericidal activity (1, 4, 8). In these experiments the bacterial populations recoverable from the laundered woolskins were greatly reduced. About a 5.0-$\log_{10}$ reduction in bacterial population was observed when woolskins contaminated with *S. aureus* by direct contact or aerosol were laundered in glutaraldehyde (5,000 ppm) in combination with nonionic detergent (265 ppm; Table 2). Increasing the concentration of glutaraldehyde did not increase the efficacy of the laundering procedure when a nonionic detergent was employed (Table 2). Glutaraldehyde (20,000 or 40,000 ppm) in combination with the anionic detergent (265 ppm) also appeared to be a relatively effective combination of disinfectant and detergent (Table 2).

Laundering of woolskins contaminated with *P. aeruginosa* in glutaraldehyde (40,000 ppm) and nonionic or anionic detergent (265 ppm) reduced the bacterial population on the wool or leather 3.0 to 5.0 $\log_{10}$. Concentrations of glutaraldehyde less than 20,000 ppm in combination with anionic detergent (265 ppm) were less effective (Table 3).

As noted above, processing the contaminated woolskins through the washing cycles without any added detergent or disinfectant resulted in partial reductions of the bacterial populations of the woolskins. Although viable bacteria were not

TABLE 2. *Effect of laundering with detergents and with detergent-disinfectant combinations on the population density of Staphylococcus aureus (ATCC 6538) placed on woolskins by direct contact or aerosol*[a]

Detergent[b]		Disinfectant[c]		Log_{10} reduction in population density[d]			
				Direct contact		Aerosol	
Type	Concn (ppm)	Type	Concn (ppm)	Wool	Leather	Wool	Leather
None	265	None		2.1	1.7		
Nonionic	265	None		1.4	0.9	3.3	0.8
Anionic	265	None		2.1	0.9	1.9	1.1
Nonionic	265	Quaternary	60	2.1	2.8	3.9	1.5
Nonionic	265	Quaternary	120	2.1	0	3.3	1.2
Nonionic	265	Phenolic	1,000	3.4	0.1	5.2	0.6
Nonionic	265	Phenolic	2,000	>5.1	2.0	>4.7	0.7
Nonionic	265	Glutaraldehyde	5,000	4.7	4.9	5.3	5.2
Nonionic	265	Glutaraldehyde	10,000	4.8	5.0	4.8	4.6
Nonionic	265	Glutaraldehyde	20,000	>4.1	>4.5	>4.3	>3.6
Nonionic	265	Glutaraldehyde	40,000	>5.0	>3.3	>3.0	>2.4
Anionic	265	Quaternary	60	4.6	0.3	4.7	0.4
Anionic	265	Quaternary	120	3.1	0.2	4.9	1.4
Anionic	265	Phenolic	1,000	4.5	0.2	>4.6	3.1
Anionic	265	Phenolic	2,000	>3.9	2.1	5.3	0.8
Anionic	265	Glutaraldehyde	20,000	3.7	>4.0	3.0	>2.5
Anionic	265	Glutaraldehyde	40,000	>4.7	>4.9	>4.3	>3.1
Detergent-sanitizer	665			1.6	0.6	4.2	1.9
Detergent-sanitizer	1,330			2.8	0.7	4.2	1.9

[a] Bacteria were not detected in the rinse water (<1 viable organism/5 ml).

[b] Nonionic detergent was an alkylaryl polyether alcohol. Anionic detergent was a sodium alyklaryl polyether sulfate. The detergent-sanitizer consisted of the lauryl pyridinium salt of 5-chloro-2-mercaptobenzothiazole (0.20%), 2-2-methylenebis (3,4,6-trichlorophenol) (0.05%), brominated isomers of salicylanilide "typically composed of" 95% 3,5,4'-tribromosalicylanilide, and 5% "related isomers" (0.35%).

[c] The quaternary ammonium disinfectant consisted of *n*-alkyl(C_{14}, C_{12}, C_{16})dimethylbenzylammonium chloride (80%), ethyl alcohol, and water. The phenolic disinfectant was orthobenzylparachlorophenol ($C_{13}H_{11}OCl$). The glutaraldehyde disinfectant was supplied as a 50% aqueous solution.

[d] Log_{10} [bacteria per square inch of control (unlaundered) sample/bacteria per square inch of test (laundered) sample].

usually detected in the rinse water from the second rinse (Tables 2 and 3), bacteria were still recoverable from the processed samples in large numbers. This implies that the partial reduction of the bacterial populations on the woolskins were primarily due to elution of bacteria from woolskins. This suggests that the physical factors of the laundering process do not in themselves produce a bactericidal effect.

The method of exposure of the woolskins to bacteria apparently had a limited effect on the degree of bacterial population reduction. For instance, laundering woolskins contaminated by exposure to an aerosol of *P. aeruginosa* in either nonionic or anionic detergent did affect about a 5.0-log_{10} reduction of the bacterial population on the wool as compared to a 1.0-log_{10} reduction of the bacterial population on the wool when the shearlings were contaminated by the direct contact method (Table 3). As noted above, in experiments employing the combination detergent-sanitizer, the laundered wool side of the woolskins contaminated by exposure to aerosols appeared to exhibit a greater degree of population reduction than when contaminated by direct contact (Tables 2 and 3). Generally, the samples exposed to an aerosolized culture of bacteria received less bacteria than those exposed by direct contact. The leather particularly had a lower initial bacterial population when exposed to the bacterial aerosols; this would be expected, since the thick covering of wool would make it difficult

TABLE 3. *Effect of laundering with detergents and with detergent-disinfectant combinations on the population density of Pseudomonas aeruginosa (ATCC 15442) placed on woolskins by direct contact or aerosol*

Detergent[a]		Disinfectant[b]		Log_{10} reduction in population density[c]				Recoverable bacteria in rinse water/ml	
				Direct contact		Aerosol			
Type	Concn (ppm)	Type	Concn (ppm)	Wool	Leather	Wool	Leather	Direct contact	Aerosol
None	265	None		1.2	0.5			ND[d]	ND
Nonionic	265	None		1.1	0	4.9	0	ND	ND
Anionic	265	None		1.1	1.0	4.6	2.5	ND	ND
Nonionic	265	Quaternary	60	2.8	1.1	5.1	1.0	ND	9.0×10^1
Nonionic	265	Quaternary	120	2.8	0.6	4.0	2.7	ND	5.5×10^1
Nonionic	265	Phenolic	1,000	5.0	3.4	4.8	1.3	ND	ND
Nonionic	265	Phenolic	2,000	5.2	1.6	4.1	<0.1	8.8×10^1	7.3×10^1
Nonionic	265	Glutaraldehyde	20,000	4.9	4.1	4.9	1.5	ND	ND
Nonionic	265	Glutaraldehyde	40,000	>5.2	>5.1	5.2	>4.4	ND	ND
Anionic	265	Quaternary	60	5.4	0.1	4.9	1.0	2.2×10^3	3.0×10^3
Anionic	265	Quaternary	120	4.4	2.1	5.1	1.4	6.6×10^1	3.4×10^1
Anionic	265	Phenolic	1,000	4.9	3.5	>4.8	3.5	8.6×10^1	7.7×10^1
Anionic	265	Phenolic	2,000	4.3	1.6	4.4	1.3	1.5×10^1	1.7×10^1
Anionic	265	Glutaraldehyde	5,000	4.3	3.5	1.4	1.2	ND	ND
Anionic	265	Glutaraldehyde	10,000	3.6	3.2	4.4	2.3	ND	ND
Anionic	265	Glutaraldehyde	20,000	>5.3	>4.2	>4.8	2.6	ND	ND
Anionic	265	Glutaraldehyde	40,000	>5.4	>4.2	>4.6	>2.7	ND	ND
Detergent-sanitizer	665			2.7	1.2	3.1	0.6	ND	ND
Detergent-sanitizer	1,330			2.4	0.7	4.8	2.6	3.1×10^1	3.1×10^1

[a] Nonionic detergent was an alkylaryl polyether alcohol. Anionic detergent was a sodium alkylaryl polyether sulfate. The detergent-sanitizer consisted of the lauryl pyridinium salt of 5-chloro-2-mercaptobenzothiazole (0.20%), 2-2-methylenebis(3,4,6-trichlorophenol) (0.05%), brominated isomers of salicylanilide "typically composed of" 95% 3,5,4'-tribromosalicylanilide, and 5% "related isomers" (0.35%).

[b] The quaternary ammonium disinfectant consisted of *n*-alkyl(C_{14}, C_{12}, C_{16})dimethylbenzylammonium chloride (80%), ethyl alcohol, and water. The phenolic disinfectant was ortho-benzyl-para-chlorophenol ($C_{13}H_{11}OCl$). The glutaraldehyde disinfectant was supplied as a 50% aqueous solution.

[c] Log_{10} [bacteria per square inch of control (unlaundered) sample/bacteria per square inch of test (laundered) sample].

[d] ND = Bacteria not detected in the rinse water (<1 viable organism/5 ml).

for bacteria to penetrate to the leather. The samples were exposed to the aerosol wool side up to simulate in-use situations.

The efficacy of the combinations of detergent and glutaraldehyde, quaternary ammonium disinfectant, and phenolic disinfectant, and combination detergent-sanitizer, were not generally improved by increasing the disinfectant concentration. However, in certain experiments with particular disinfectants there appeared to be some advantage in employing an increased concentration of disinfectant. For instance, when woolskins were contaminated with *S. aureus* by direct contact and laundered in the phenolic disinfectant (1,000 ppm) plus nonionic detergent (265 ppm), the bacterial population on the wool and leather was reduced 3.4 and 0.1 log_{10}, respectively. When the phenolic disinfectant concentration was increased to 2,000 ppm, the degree of bacterial population reduction on the wool was 5.1 log_{10} and 2.0 log_{10} on the leather (Table 2). The effect of different concentrations of glutaraldehyde on the efficacy of the laundering procedure has been discussed above.

The *p*H at which the woolskins were laundered was relatively similar in all experiments. The final rinse water after addition of sour was acidic (*p*H 6); the wool and the leather of the laundered

samples were slightly basic (*p*H 7.5). When glutaraldehyde was used, the wash water was made alkaline at the start of the wash cycle (*p*H 9) since several investigators have reported that this disinfectant is most effective at an alkaline *p*H (1, 4, 8).

The most effective detergent-disinfectant observed in this study was glutaraldehyde plus the nonionic detergent. Glutaraldehyde, at the highest concentrations used, altered the texture of the wool and leather, apparently from the precipitation of either anionic or nonionic detergents, as it did in the virus studies recently reported by Sidwell et al. (7). These undesirable effects were reduced by lowering the glutaraldehyde concentration to 5,000 ppm. However, this disinfectant at the lower concentrations was less effective in reducing the *P. aeruginosa* populations.

Bacterial populations were significantly reduced in all experiments, but only laundering in glutaraldehyde in combination with either detergent resulted in maximum removal of bacteria. Viable bacteria were usually not detected in the rinse water (<1 viable organism/5 ml).

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LITERATURE CITED

1. Borick, P. M., F. H. Dondershine, and V. L. Chandler. 1964. Alkalinized glutaraldehyde, a new antimicrobial agent. J. Pharm. Sci. 53:1273–1275.
2. Dixon, G. J., R. W. Sidwell, and E. McNeil. 1966. Quantitative studies of fabrics as disseminators of viruses. II. Persistence of poliomyelitis virus on cotton and wool fabrics. Appl. Microbiol. 14:183–188.
3. Happich, W. F., M. L. Happich, W. Windus, W. E. Palm, and J. Naghski. 1964. The tanning of shearlings with glutaraldehyde. J. Amer. Leather Chem. Ass. 59:448–461.
4. Rubbo, S. D., J. F. Gardner, and R. L. Webb. 1967. Biocidal activities of glutaraldehyde and related compounds. J. Appl. Bacteriol. 30:78–87.
5. Sidwell, R. W., G. J. Dixon, and E. McNeil. 1966. Quantitative studies on fabrics as disseminators of viruses. I. Persistence of vaccinia virus on cotton and wool fabrics. Appl. Microbiol. 14:55–59.
6. Sidwell, R. W., G. J. Dixon, L. Westbrook, and E. A. Dulmadge. 1969. A procedure for the evaluation of the virucidal effectiveness of an ethylene oxide gas sterilizer. Appl. Microbiol. 17:790–796.
7. 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 on polio and vaccinia viruses. Appl. Microbiol. 19:53–59.
8. Stonehill, A. A., S. Krop, and P. M. Borick. 1963. Buffered glutaraldehyde, a new chemical sterilizing solution. Amer. J. Hosp. Pharm. 20:458–465.
9. Wilkoff, L. J., L. Westbrook, and G. J. Dixon. 1969. Factors affecting the persistence of *Staphylococcus aureus* on fabrics. Appl. Microbiol. 17:268–274.
10. Wilkoff, L. J., L. Westbrook, and G. J. Dixon. 1969. Persistence of *Salmonella typhimurium* on fabrics. Appl. Microbiol. 18:256–261.