Factors Affecting the Persistence of *Staphylococcus aureus* on Fabrics

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The persistence of *Staphylococcus aureus* (Smith) on wool blanket, wool gabardine, cotton sheeting, cotton knit jersey, cotton terry cloth, and cotton wash-and-wear fabrics was studied. The fabrics were exposed to bacterial populations by three methods: direct contact, aerosol, and a lyophilized mixture of bacteria and dust having a high content of textile fibers. The contaminated fabrics were held in 35 or 78% relative humidities at 25 C. In general, the persistence time of *S. aureus* populations on fabrics held in 35% relative humidity was substantially longer when the fabrics were contaminated by exposure to aerosolized cultures or to dust containing bacteria than when contaminated by direct contact. In a 78% relative humidity, bacterial populations on the fabrics persisted for substantially shorter periods of time regardless of the mode of contamination or fabric type. Cotton wash-and-wear fabric (treated with a modified triazine resin) was the material on which populations of *S. aureus* persisted for the shortest time. This organism retained its virulence for Swiss mice after being recovered from wool gabardine swatches held 4 weeks in 35% relative humidity and 6 weeks in 78% relative humidity.

*Staphylococcus aureus* is frequently found on normal human skin and mucous membranes. The organism commonly occurs in the anterior nares and on the dorsum of the hands (12). The dynamics of acquisition are not fully understood, but several important observations have been made. Newborn children rapidly acquire *S. aureus*, and in nurseries the strain carried is the one that is predominant within the hospital unit (1). Dissemination of the organism in the nursery is primarily by direct contact with personnel, although it is known that *S. aureus* can survive in fomites (13). As with newborns, the most important route of spread of this organism among adults is probably direct contact, but airborne dissemination and contamination of bed clothing may also be involved (6).

In an effort to elucidate the possible role of fabrics as disseminators of *S. aureus*, we have studied the persistence of this organism on a variety of wool and cotton fabrics. The fabrics were exposed to the microorganism by direct contact, aerosol, and dust containing bacteria; they were held in relative humidities of 78 and 35% at a temperature of 25 C. The number of viable bacterial cells was determined as a function of time and expressed as the number of bacterial cells recovered per 6.45 cm² of fabric.

**MATERIALS AND METHODS**

**Organism and culture conditions.** A lyophilized culture of *S. aureus* (Smith (19)) was obtained from Parke, Davis & Co., Detroit, Mich. The virulence of this strain for mice is increased when the organisms are suspended in 5% hog-gastric mucin and inoculated intraperitoneally (ip; M. Fisher, Parke, Davis & Co., personal communication).

The lyophilized culture was suspended in Trypticase Soy Broth (BBL), grown for about 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 sample was thawed rapidly; 1.0 ml was inoculated into a flask containing Trypticase Soy Broth, and the culture was incubated for 18 hr at 37 C with gentle agitation. A cell density of about $1.5 \times 10^9$ cells/ml was usually obtained.

**Fabrics.** Fabrics used in these studies were wool blanket, wool gabardine, cotton sheeting, cotton knit jersey, cotton terry cloth, and cotton wash-and-wear. The cotton wash-and-wear material was treated with a modified triazine resin (Perma Fresh 197) to impart wash-and-wear properties to the fabric (J. W. Richardson, Russell Mills, Inc., Alexander City, Ala., personal communication). All fabrics had been bleached, were undyed, and were not impregnated with antimicrobial or moth-proofing substances. Complete descriptions of these fabrics have been reported elsewhere (18). Each of the fabrics was cut into circular swatches 5.08 cm in diameter with a mechanized die.
Humidity maintenance. A saturated solution of sodium chloride was used to produce a relative humidity of about 78%, and dry potassium acetate was used to produce a relative humidity of approximately 35%. Air-tight, insulated cabinets held in a 25-C incubator room were used for fabric storage to maintain the desired temperature and humidity.

Sterilization of materials. All fabrics and plastic materials were sterilized with ethylene oxide (Steri-Vac Gas Sterilizer, Minnesota Mining and Mfg. Co., St. Paul, Minn.) at approximately 30 C for 180 min.

Methods of exposure of fabrics to S. aureus. Fabric swatches were exposed to the organism in three ways: by direct contact, by aerosol, or by dust. For the direct contact method, 0.4 ml of a standardized bacterial suspension (an 18-hr culture adjusted to a cell density of 2 × 10^8 cells/ml) was pipetted onto the fabric. Exposure to aerosol was carried out in a molded plastic isolator (Germfree Laboratories, Inc., Miami, Fla.). The aerosol was produced by two DeVilbis atomizers facing each other and separated by a wire grid holding the fabric swatches. A total pressure of 13 lb/in^2 of nitrogen gas was applied to these two atomizers. The atomizers produced particles of which 95% were from 0.27 to 6.0 μm in diameter at a density of about 2.1 × 10^6 particles/liter, as determined by an aerosol photometer (20). The bacteria-containing fog was allowed to settle on the swatches for 1 hr prior to removal from the chamber.

To expose the swatches to dust containing bacteria, a sterile household dust was obtained which was composed predominantly of textile fibers (18). This material was added to an 18-hr broth culture of S. aureus and lyophilized. The fabrics were exposed to this lyophilized mixture of dust and bacteria by sealing the swatches in a large battery jar with the dust-containing bacteria mixture and giving the closed jar a random rotating, tumbling action for 15 min (18). The swatches were then removed and placed in the specified humidities. Bacterial assays on randomly selected bacterial-contaminated swatches of all fabrics indicated that all swatches received essentially the same quantity of recoverable bacteria.

Method for determination of bacterial persistence on fabrics. Seventy-five swatches of each fabric were exposed to the organism by one of the three methods described above. The swatches were placed five to a dish in elevated-lid plastic petri dishes (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.). The swatches were separated in the dish by sterile, glass-fiber screens and the dishes were then stored at the desired humidity. The bacterial population density on five swatches was determined at zero-time (immediately after exposure to bacteria) and at the following time intervals: 2 hr, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 weeks after exposure of the swatches to the organism, or at least two time intervals after bacteria could no longer be recovered from the fabric.

The bacterial population density on each fabric was determined by cutting each swatch into small pieces with sterile scissors and macerating with 25 ml of physiological saline in a Sorvall Omnimix homogenizer (Ivan Sorvall, Inc., Norwalk, Conn.). The material was then serially diluted tenfold from 10 (undiluted) to 10^-4 with physiological saline. The number of viable bacteria was determined by plating 0.1 or 0.2 ml amounts of each dilution on tryptic soy agar plates. The plates were inverted and incubated at 37 C for 24 hr, and the number of bacterial colonies were counted. The results are expressed as the number of bacteria recovered per 6.54 cm^2 of fabric swatch.

Determination of virulence of bacteria recovered from swatches. In several persistence experiments, the virulence of bacteria recovered from wool gabardine fabric swatches held for several weeks in 78 or 35% relative humidity at 25 C was determined. Bacterial colonies were randomly picked from plates made from the swatches. Trypticase Soy Broth cultures were inoculated and incubated at 37 C with gentle agitation for 18 to 24 hr. The broth cultures were then titrated in Swiss mice by a series of 1.0 log₁₀ dilutions in 5% gastric mucin. Samples (0.1 ml) were inoculated into a group of 10 mice for each dilution. The number of viable cells per milliliter of culture was determined by plating a series of dilutions of the broth culture. The LD₅₀ is defined as the number of cells required to kill 50% of 8- to 10-g Swiss mice within 15 days after infection with cultures of S. aureus, as determined by the method of Reed and Muench (15). For comparison, the LD₅₀ of a fresh culture of S. aureus was also determined.

RESULTS

The results obtained from each group of five swatches tested at the various time intervals are presented in Fig. 1–6. The number of bacteria

![Fig. 1. Persistence of S. aureus (Smith) on wool blanket material held at 25 C in two humidities. Data expressed as the mean number of bacteria recovered per 6.54 cm² of fabric swatch. The 95% confidence limits were usually less than ± 1 log₁₀ from the mean. Method of exposure to bacteria: solid line, direct contact; dashed line, aerosol; dotted line, lyophilized mixture of dust and bacteria.](http://aem.asm.org/Downloaded_from_October_18_2017_by_guest.png)
recovered per 6.54 cm² of each individual swatch varied relatively little (for this type of biological work) from the mean, regardless of the manner of exposure of the swatch; the 95% confidence limits were usually less than ±1.0 log₁₀ from the mean. Each method of exposure of swatches to bacteria resulted in approximately the same population density (10⁴ to 10⁶ bacterial cells per 6.54 cm² of fabric) recoverable at zero-time.

In general, the persistence time of bacterial populations on fabrics held in 35% relative humidity was substantially longer when the fabrics were contaminated by exposure to aerosolized cultures or to dust containing bacteria than when contaminated by direct contact. For example, fabric swatches of wool blanket, wool gabardine, cotton sheeting, cotton knit jersey, and cotton terry cloth which had been exposed to an aerosol of the organism and held in a relative humidity

![Fig. 2. Persistence of S. aureus (Smith) on wool gabardine material held at 25 C in two humidities. Symbols expressed as in Fig. 1.](image)

![Fig. 3. Persistence of S. aureus (Smith) on cotton sheeting material held at 25 C in two humidities. Symbols expressed as in Fig. 1.](image)

![Fig. 4. Persistence of S. aureus (Smith) on cotton knit material held at 25 C in two humidities. Symbols expressed as in Fig. 1.](image)

![Fig. 5. Persistence of S. aureus (Smith) on cotton terry cloth material held at 25 C in two humidities. Symbols expressed as in Fig. 1.](image)

of 35% retained viable bacterial populations for 17 to 24 weeks (Fig. 1–5). In the same relative humidity, swatches of wool blanket, wool gabardine, cotton knit jersey, cotton terry cloth, and cotton wash-and-wear fabrics exposed to a lyophilized mixture of dust and bacteria retained viable bacterial populations for 14 to 24 weeks (Figs. 1, 2, 4–6). On the other hand, bacterial populations persisted for only 1 to 6 weeks on fabric swatches exposed by direct contact and held in this relative humidity (Fig. 1–6).

The higher relative humidity of 78% appeared to provide a less favorable environment for the persistence of this organism. In this relative humidity, bacterial populations generally persisted for substantially shorter periods of time, regardless of the mode of contamination or fabric type. In fact, in a 78% relative humidity, only
wool gabardine fabric that had been exposed to a lyophilized mixture of dust and bacteria retained viable bacterial populations for as long as 10 weeks (Fig. 2).

Populations of S. aureus persisted for the shortest time on cotton wash-and-wear fabric which had been treated with a modified triazone resin (with the exception of swatches exposed to dust containing bacteria and held in 35% relative humidity; Fig. 6). Daily observations of the persistence of viable bacterial populations on swatches of this fabric exposed by direct contact and held in 35 or 78% relative humidity revealed that the bacterial populations persisted less than one day (Fig. 7). For comparison, daily observations were made of the persistence of S. aureus populations on swatches of cotton sheeting contaminated by direct contact; the data indicate that the persistence time in 35 or 78% relative humidity was about 6 to 7 days (Fig. 8).

The virulence of S. aureus recovered from wool gabardine swatches held for 4 weeks in 35% relative humidity and for 6 weeks in 78% relative humidity was determined in 8- to 10-g Swiss mice. The LD₀ of an 18- to 24-hr culture of organisms recovered from swatches held in 35% relative humidity was $4.8 \times 10^4$ cells per animal, which represented about a 30% decrease in virulence when compared with the LD₀ of a fresh 18- to 24-hr culture of S. aureus ($2.4 \times 10^4$ cells per animal). When organisms were recovered from swatches held for 6 weeks in 78% relative humidity, the LD₀ of an 18- to 24-hr culture was $7.4 \times 10^6$ cells per animal, which was a decrease in virulence of about 99.7%.

DISCUSSION

Langmuir has pointed out that microorganisms can be spread by contact, droplet, droplet nuclei (residues resulting from evaporation of moisture from droplets), and by dust (7). In our study, three of these methods were used to investigate the survival and persistence of S. aureus (Smith) on different types of fabric swatches. Factors which can influence the survival and infectivity of microorganisms on fabrics include genetic differences between strains of bacteria, media in which the microorganisms are suspended, temperature, relative humidity, light, fiber type, fabric construction and finish, and physiological characteristics of the microorganism (10). For instance, Church and Loosli found that S. aureus and S. salivarius which had been suspended in 1% mucin, aerosolized, and allowed to settle on sterile sheets survived subsequent drying and ironing in greater numbers than when the suspending medium was water or broth (2). In our study, all of the variables investigated, i.e., fabric type and construction, mode of exposure and relative
humidity, appear to be of importance in the persistence of *S. aureus* on fabrics.

Fabric type is an important factor in influencing the duration of persistence of bacteria in and on textiles and clothing (11). The physical characteristics of the fibers themselves, such as the scales of the wool fibers and the twisted tubular cellulose fibers of cotton, undoubtedly influence the attachment of the bacteria. It is also possible that electric charges on the surfaces of both the fiber and the bacterial cell may be important in influencing the attachment of bacteria to the fabric surface. In addition, the fabric construction, such as type of yarn and tightness of weave, the textile processing of the fabric, and the moisture content of the fabric are probably factors which influence the viability of bacteria found in and on textiles.

In general, fabric swatches contaminated by an aerosol or by a lyophilized mixture of dust and bacteria and held in 35% relative humidity retained viable organisms for substantially longer periods of time than when these swatches were held in 78% relative humidity (Fig. 1–5). Swatches of cotton sheeting contaminated by a lyophilized mixture of dust and bacteria appeared to be an exception, since bacterial persistence on these swatches was about the same (6 to 8 weeks) in either relative humidity (Fig. 3). When swatches were contaminated by direct contact, the periods of persistence on fabrics in the two relative humidities were comparatively brief (Fig. 1–5). The difference in persistence time when bacterial populations were applied to the fabric swatches by direct contact or by aerosol and held in 35% relative humidity is interesting, since the fabric swatches which were contaminated by aerosol consistently retained viable bacterial populations for significantly longer periods of time (except for cotton wash-and-wear fabric; Fig. 6). The population densities of the swatches recoverable at zero-time and at 2 hr after exposure of the swatches were about the same for both methods (Fig. 1–5). Therefore, neither method of application produced an immediate effect upon the viability of the bacterial populations. Perhaps aerosolization of the cultures produced a monodispersed population of bacterial cells which resulted in a distribution of the cells on the surface of the swatches such that each cell had its own individual environment. When cells were applied to the swatches by direct contact, we assumed that the broth cultures were monodispersed because they were thoroughly agitated; however, some degree of clumping could have occurred and two or more cells might have been deposited in the same environment on the fabric surface. If this did occur, then these cells would have to compete with each other for survival on the surface of the fabric.

The persistence of *S. aureus* on the cotton wash-and-wear swatches was relatively brief (Fig. 6, 7), except when the fabric was contaminated by a lyophilized mixture of dust and bacteria and held in 35% relative humidity (Fig. 6). In this case, the organism persisted for 24 weeks. The cotton wash-and-wear material had been treated with a modified triazone resin (Perma Fresh 197) to impart wash-and-wear properties to the fabric. This increased persistence may have been due to lack of moisture, thus not giving the resin an opportunity to come into direct contact with the dry bacteria.

McNeil and Greenstein have reported that populations of *S. aureus* persisted on wool blanket fabric for 100 days and on cotton sheeting fabric for 54 days in a 70% relative humidity (11). When these fabrics were held in 28% relative humidity, the length of persistence was 127 and 84 days, respectively. They employed a method for contaminating the fabrics which was similar to the direct contact method used in the present study.
In our study, the bacterial population of wool blanket and cotton sheeting fabrics contaminated by direct contact and held in 78 or 35% humidity persisted for relatively brief periods of time (Fig. 1, 3). However, when the fabrics were contaminated by an aerosol of S. aureus or by a lyophilized mixture of dust and bacteria and held in 35% relative humidity, the bacterial populations persisted for periods of time comparable to the duration of persistence observed by McNeil and Greenstein (11).

Lidwell and Lowbury, in a study on the survival of bacteria in dust, included the effects of relative humidity and light (9). They found that the death rate of most bacteria increased with increase of humidity. They also found that exposure to daylight, low intensity ultraviolet radiation, or fluorescent lights increased the death rate to approximately five times that obtained in the dark, if the relative humidity was maintained at 60%. At lower relative humidities, the antibacterial action was considerably slower. Crosbie and Wright found that Corynebacterium diphtheriae remained viable in dust for 102 days (3). In our study, fabrics contaminated with S. aureus in dust and held in 35% relative humidity retained viable bacterial populations for 8 to 24 weeks, depending upon the particular type of fabric used (Fig. 1–6).

S. aureus was still infectious for Swiss mice after recovery from wool gabardine swatches. Similar results on infectivity have been obtained by Garrod who contaminated dust with hemolytic streptococci and found that the organism was still infectious for mice at the end of ten weeks (5).

The data in our study indicate that populations of S. aureus can survive on fabrics for sufficient periods of time to be of epidemiological importance. The most probable route of dispersion of S. aureus is by contamination of the skin from the upper respiratory tract, perhaps by hand contact; then particles of desquamated skin are dispersed into the air either directly, as a result of skin movements, or after transfer from the skin to clothing or other textiles (8). Noble has reported that the bedclothes of staphylococci carriers in hospitals become rapidly contaminated (14). Rubbo et al., reporting on the dispersion of staphylococci from textiles, concluded that the numbers of airborne organisms dispersed were not correlated with the numbers of airborne wool fibers from blankets, but that they were associated with considerably smaller particles which they suggested might consist of cotton fibers, the dominant component in the fine dust of the ward air, or "free" organisms (16). They also made the observation that friction between two textiles appeared to be the most effective mechanism of aerial dispersion (17). Davies and Noble reported that microscopic examination of airborne particles from a hospital ward showed that many skin scales were present (4). They also showed that the rise in bacterial content of the air during bedmaking was closely paralleled by the rise in the numbers of airborne skin scales, and they were able to obtain photomicrographs showing colonies of cocci growing on skin scales collected from the air and incubated on nutrient agar plates. Williams points out that in hospitals there is often widespread contamination of bedding and floor dust with S. aureus (21). He states that staphylococci can remain viable, in blankets at least, for some time and are redispersed into the air.

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LITERATURE CITED


