

# Survival of Enterococci and Staphylococci on Hospital Fabrics and Plastic

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**The transfer of gram-positive bacteria, particularly multiresistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), among patients is a growing concern. One critical aspect of bacterial transfer is the ability of the microorganism to survive on various common hospital surfaces. The purpose of this study was to determine the survival of 22 gram-positive bacteria (vancomycin-sensitive and -resistant enterococci and methicillin-sensitive and -resistant staphylococci) on five common hospital materials: smooth 100% cotton (clothing), 100% cotton terry (towels), 60% cotton–40% polyester blend (scrub suits and lab coats), 100% polyester (privacy drapes), and 100% polypropylene plastic (splash aprons). Swatches were inoculated with  $10^4$  to  $10^5$  CFU of a microorganism, assayed daily by placing the swatches in nutritive media, and examining for growth after 48 h. All isolates survived for at least 1 day, and some survived for more than 90 days on the various materials. Smaller inocula ( $10^2$ ) survived for shorter times but still generally for days. Antibiotic sensitivity had no consistent effect on survival. The long survival of these bacteria, including MRSA and VRE, on commonly used hospital fabrics, such as scrub suits, lab coats, and hospital privacy drapes, underscores the need for meticulous contact control procedures and careful disinfection to limit the spread of these bacteria.**

Infections, particularly those caused by antibiotic-resistant gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), are a growing concern, particularly in units in which patients are immunosuppressed either intentionally (as for transplantation) or as a result of trauma (severe burns) or disease (such as acquired immunodeficiency disease). As more bacteria become resistant to antibiotics, our ability to control the spread of these bacteria with antibiotic treatments decreases.

The environment can play a marked role in the nosocomial transmission of microorganisms. Last year, a hospital outbreak of MRSA was directly linked to a stretcher and a handheld shower (1), and an electronic ear-probe thermometer was implicated in an outbreak of VRE (10). Also, admission of a VRE-free patient to a hospital room recently occupied by a VRE-colonized patient was found to be an independent risk factor for nosocomial acquisition of VRE by the previously uncolonized individual (7).

In addition, garments of health care workers are an important aspect of the environment that can easily become contaminated. A recent study reported that 65% of nurses who had performed patient care activities on patients with MRSA in a wound or urine contaminated their nursing uniforms or gowns with MRSA (4).

One critical factor for transmission of a microorganism from a person (patient or health care worker) to the environment and then to another person is the ability of that microbe to survive on that environmental surface. A few studies have examined the survival of gram-positive bacteria on various surfaces, such as glass (9), aluminum foil (5), polyvinyl chloride (16), countertops (3, 8), and bed rails and stethoscopes (8).

Few studies have examined the viability of gram-positive bacteria on fabrics, and those that have tested survival of staphylococci primarily on cotton (2, 12, 17). However, there are many other garment materials and fabrics, especially synthetics and cotton-synthetic blends, that are used more often than cotton in hospitals today. Also, with increasing concerns about VRE, enterococcal survival on fabrics must be considered. Therefore, the purpose of this study was to examine systematically the survival of several clinical and environmental staphylococcal and enterococcal isolates on fabrics and plastic commonly used in hospitals.

## MATERIALS AND METHODS

**Microorganisms.** All microorganisms were recently isolated from patients or hospital environmental surfaces. The isolates tested included four *Enterococcus faecalis* isolates (two vancomycin sensitive, one VRE *vanA*, and one VRE *vanB*), four *Enterococcus faecium* isolates (two vancomycin sensitive, one VRE *vanA*, and one VRE *vanB*), one *Enterococcus casseliflavus* isolate (VRE *vanC*), one *Enterococcus gallinarum* isolate (VRE *vanC*), six coagulase-negative staphylococci (CNS) (three methicillin sensitive and three methicillin resistant), and six *S. aureus* isolates (three methicillin sensitive and three methicillin resistant). The *S. aureus* isolates were tested for antibiotic sensitivity to 15 antibiotics using the Vitek GPS-101 susceptibility card (bioMerieux Vitek, Inc, Hazelwood, Mo.). The three methicillin-sensitive *S. aureus* (MSSA) isolates were resistant to less than three antibiotics per isolate, while the three MRSA isolates were resistant to more than six antibiotics per isolate. Hence, in this study, the MRSA can also be considered multiresistant *S. aureus*.

Isolates were grown overnight at 37°C in brain heart infusion broth (Becton Dickinson, Cockeysville, Md.). Cell density was adjusted to  $10^9$  CFU/ml using a spectrophotometer and diluted with saline to give the desired concentration to inoculate the fabric swatches. Actual bacterial counts in each inoculum were determined by serially diluting and plating the samples onto 5% TSA II plates (Becton Dickinson).

**Test materials.** Microbial survival was tested on the following materials, all of which are common in our hospital: 100% cotton (clothing), 100% cotton terry (towels and wash cloths), 60% cotton–40% polyester blends (scrub suits, lab coats, and clothing), 100% polyester (privacy curtains and clothing), and 100% polyethylene plastic (splash aprons).

**Survival test.** Swatches (0.8 cm<sup>2</sup>) of fabric and plastic were gas sterilized and properly aerated. All experiments were set up and left in a biosafety hood. Swatches were lined up in rows next to, but not touching, each other. All five materials for one microorganism were lined up in the same area of the hood.

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TABLE 1. Survival of staphylococci and enterococci on fabrics and plastic<sup>a</sup>

| Microorganism           | No. of isolates tested | Resistance characteristic <sup>b</sup> | Survival (no. of days) of individual isolates on: |            |            |            |              |
|-------------------------|------------------------|--|---|------------|------------|------------|--------------|
|                         |                        |  | Cotton  | Terry      | Blend      | Polyester  | Polyethylene |
| CNS                     | 3                      | MS                                     | 8, 16, 21   | 6, 21, 27  | 6, 6, 7    | 7, 10, 16  | 41, 51, 74   |
| CNS                     | 3                      | MR                                     | 14, 18, 20  | 14, 15, 16 | 20, 22, 28 | 16, 20, 22 | 47, >90, >90 |
| <i>S. aureus</i>        | 3                      | MS                                     | 4, 5, 19  | 9, 9, 24   | 1, 9, 21   | 10, 12, 56 | 22, 48, >90  |
| <i>S. aureus</i>        | 3                      | MR                                     | 4, 5, 21  | 2, 6, 14   | 1, 3, 3    | 1, 16, 40  | 40, 48, >51  |
| <i>E. faecalis</i>      | 2                      | VS                                     | 11, 33  | 21, 29     | 19, 29     | >90, >90   | >90, >90     |
| <i>E. faecalis</i>      | 2 <sup>c</sup>         | VR                                     | 18, 22  | 20, 22     | 18, 22     | 73, >80    | >80, >80     |
| <i>E. faecium</i>       | 2                      | VS                                     | 22, >90   | 33, >90    | 29, >90    | 43, >90    | 68, >90      |
| <i>E. faecium</i>       | 2 <sup>c</sup>         | VR                                     | 62, >90   | >80, >90   | 52, >90    | >80, >90   | >80, >90     |
| <i>E. gallinarum</i>    | 1 <sup>d</sup>         | VR                                     | 28  | 34         | 34         | >90        | >90          |
| <i>E. casseliflavus</i> | 1 <sup>d</sup>         | VR                                     | 15  | 28         | 15         | >90        | >90          |

<sup>a</sup> Mean inoculum (± standard deviation) of 4.1 (±4.4) × 10<sup>5</sup> CFU.

<sup>b</sup> Abbreviations: MS, methicillin sensitive; MR, methicillin resistant; VS, vancomycin sensitive; VR, vancomycin resistant.

<sup>c</sup> One *vanA* isolate and one *vanB* isolate.

<sup>d</sup> One *vanC* isolate.

During the 3-month period of the study, the hood fan was left on, temperature ranged from 22.9 to 24.5°C, and humidity ranged from 30 to 49%. Using an Eppendorf pipette, swatches were inoculated with 10-µl aliquots of solutions with the desired concentration of the specific microorganism. Immediately after inoculation, every hour after for the first 8 h and each day after the first day, a single swatch of each material was picked up with sterile forceps and placed into a tube of liquid thioglycolate medium (Becton Dickinson). Tubes were incubated at 37°C for 48 h and then scored for the presence (cloudy) or absence (clear) of viable bacteria. Samples from randomly chosen tubes were streaked onto 5% TSA II plates and incubated to confirm that clear tubes had no growth and that the bacteria grown from the cloudy tubes were a pure culture of the bacteria that had been used to inoculate the swatch. The majority of the time, once one swatch showed no viable bacteria, the next swatch for that sample also showed no growth. However, occasionally, a swatch taken on one day showed no growth, but the next swatch taken the next day showed viable bacteria. Therefore, we required that two consecutive swatches be negative before we considered the bacteria dead.

RESULTS

All staphylococci tested survived for at least 1 day on all fabrics and plastic (Table 1). Staphylococcal viability was longest on polyester (1 to 56 days) and on polyethylene plastic (22 to >90 days). There was a tendency for the size of the microbial inoculum to increase the survival time of the CNS tested; however, even a few hundred bacteria survived for days on most fabrics (Table 2).

The shortest survival time for any enterococcus tested was 11 days (Table 1). As with the staphylococci, the enterococci lived longer on polyester and polyethylene than on other materials. In general, enterococci lived longer than staphylococci on the fabrics and plastic. Of the enterococci, *E. faecium* tended to survive the longest on all of the surfaces tested.

TABLE 2. Effect of inoculum size on survival of CNS on fabrics and plastic

| Isolate | Inoculum (CFU)      | Survival (no. of days) on: |       |       |           |              |
|---------|---------------------|----------------------------|-------|-------|-----------|--------------|
|         |                     | Cotton                     | Terry | Blend | Polyester | Polyethylene |
| 1       | 9 × 10 <sup>2</sup> | 5                          | 11    | 9     | 6         | 16           |
| 1       | 9 × 10 <sup>4</sup> | 16                         | 21    | 6     | 10        | 41           |
| 2       | 6 × 10 <sup>2</sup> | 6                          | 6     | <1    | 9         | 6            |
| 2       | 1 × 10 <sup>5</sup> | 21                         | 27    | 7     | 16        | 51           |

DISCUSSION

Data in this study indicate that staphylococci and enterococci can survive for days to months after drying on commonly used hospital fabrics and plastic. It should be noted that survival in this study could result from a single microorganism or from many microorganisms viable at the time the sample was taken. In the future, more precise survival data could be obtained by quantitating the number of bacteria in the medium, rather than simply assessing the presence of growth versus nongrowth in that medium. Despite this methodological variation, our findings for *E. faecalis* and *E. faecium* viability on polyethylene plastic agree with the work of Wendt et al. (16) on survival of these species on polyvinyl chloride. In addition, we found extended survival for two other species, *E. gallinarum* and *E. casseliflavus*, on fabrics and plastic. Viability of enterococci on fabrics tended to be longer than their reported survival on other hospital surfaces. Specifically, Noskin et al. (8) recovered enterococci from countertops after 5 to 7 days and from bed rails at 1 day. The shorter survival times may be caused by the different surfaces tested and/or the different inocula used (Noskin et al. used 10<sup>4</sup> CFU, while we used 10<sup>5</sup> CFU). There is a report of at least 2-month survival of one VRE dried on a countertop at an undesignated concentration (3).

For staphylococci, our results are consistent with those of Wilkoff et al. (17), who reported that one *S. aureus* isolate lived 1 week on cotton and 2 weeks on terry. In contrast, Scott and Bloomfield (12) showed *S. aureus* surviving only 4 to 24 h on cloth; however, their inocula were low (10<sup>2</sup> CFU). Our limited study with CNS suggests that inoculum size can affect survival (Table 2). This conclusion is consistent with a study showing a dose-response effect on the survival of a *S. aureus* and an *E. faecalis* on aluminum foil (5). Mechanistically, the effect of inoculum concentration on cell viability is consistent with the concept of cryptic growth in which bacteria in a starving or nutrient-limiting condition can live on nutrients from dying cells nearby (15). Hence, with higher bacterial inocula, there would be more dying cells to sustain the few living bacteria longer.

How our inocula sizes (10<sup>2</sup> and 10<sup>5</sup>) relate to the numbers of bacteria encountered by health care workers probably depends upon the health care worker and the particular task they are conducting. Rutala et al. (11) counted the number of MRSA on elevated surfaces, such as countertops, in rooms of patients

with MRSA and found up to 70 MRSA/Rodac plate (approximately 3.5 MRSA/cm<sup>2</sup>) on these inanimate surfaces. When wound surfaces are examined, the microbial load can be much higher. In a study of 141 swabs of burn wounds, the median bacterial count was  $3.4 \times 10^3$  microorganisms/cm<sup>2</sup>; however, the counts ranged from 0 to  $3 \times 10^8$  bacteria/cm<sup>2</sup>. Therefore, one might postulate that in changing a dressing for an infected burn wound or a diabetic ulcer, for example, one might encounter more than the 10<sup>5</sup> bacteria/swatch that we tested, but in contacting a surface in a patient's room, a microbial density lower than the 10<sup>2</sup> bacteria/swatch might be anticipated.

There have been conflicting reports about whether antibiotic resistance affects bacterial survival. Information about enterococci is limited. Wendt et al. (16) found no difference in the viability of vancomycin-sensitive versus vancomycin-resistant *E. faecalis* and *E. faecium* dried on polyvinyl chloride. We also found that vancomycin resistance made no difference in survival for either *E. faecalis* and *E. faecium* or for *E. gallinarum* and *E. casseliflavus* when tested on another plastic (polyethylene) or when tested on four different fabrics (Table 1).

There is some information on the effect of resistance on staphylococcal survival. Duckworth et al. (6) found no difference in survival between MSSA and MRSA on formica, while Wagenvoort and Penders (14) found a single epidemic strain of MRSA that lived longer on dust than did a single hospital strain of MSSA. Beard-Pegler et al. (2), by dividing MRSA strains into a few that were very widespread or epidemic versus others that were not, demonstrated that the widespread MRSA survived longer on cotton than did either the local MRSA or hospital strains of MSSA. The nonepidemic strains of MRSA and the hospital MSSA lived equally long. The MRSA strains used in our study were regular, not epidemic, strains. Hence, our results agree with those of Beard-Pegler et al. (2) in that there was no consistent difference in survival between MRSA and MSSA inoculated onto the two cotton surfaces (smooth and terry) tested. Neither did we find significant differences in viability between MRSA and MSSA when tested on synthetic or cotton-synthetic blend fabrics or on polyethylene plastic. Also, Beard-Pegler et al. (2) reported no difference in survival based on antibiotic sensitivity of the CNS strains that they tested. Our studies confirmed these results for CNS on cotton and extended them to the blend, polyester, and polyethylene materials that we also tested.

In conclusion, data in this study indicate that staphylococci and enterococci can survive for extended periods of time on materials commonly worn by patients and health care workers and on various other fabrics in the hospital environment. While most previous studies have tested survival of principally staphylococci using cotton as a representative fabric (2, 12, 17), the present study examined the survival of enterococci, including VRE, and staphylococci, on a number of different fabrics. Most of the bacteria tested in this study survived longer on polyester than on cotton. Hence, fabric type may influence survival. The length of survival of these organisms on the various materials may have significant infection control implications. For example, the polyester tested in this study is the material used at our hospital for privacy drapes, which are handled by both patients and staff when they are drawn around the patient's bed. Staphylococci and enterococci survived for days to months on this fabric, thereby suggesting that such drapes could act as reservoirs for these bacteria. Also, all

bacteria tested survived for at least a day on the cotton-polyester blend. Since scrub suits, lab coats, and many regular clothes are blends, blends are probably the most common fabric worn by health care workers. One can easily postulate how these fabrics could become vectors for the spread of staphylococcal or enterococcal organisms as a health care worker moves from one patient to another, and the sleeve of his lab coat, for example, contacts different patients. Hence, the lengthy survival of these microorganisms on these various materials underscores the importance of both meticulous contact control procedures and thorough disinfection of hospital fabrics and plastic to minimize the spread of gram-positive microorganisms such as MRSA and VRE.

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