In vitro evaluation of a novel process for reducing bacterial contamination of environmental surfaces

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Background: Disinfection of contaminated surfaces is an integral and challenging aspect of infection prevention. We evaluated the ability of Goldshield 5 (GS; NBS Technology, Laurelton, NY), an antimicrobial surfactant that coats surfaces with covalently bound octadecyldimethylammonium ions, to reduce the bacterial burden on contaminated surfaces.
Methods: We tested the GS product for inhibitory activity against patient isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa (*PA), and *Escherichia coli* (EC) on fabric according to the garment industry standard American Association of Textile Chemists and Colorists 100 protocol. We also tested the product for activity against these same isolates in carrier tests with a modified Association of Official Analytical Chemists use-dilution method.
Results: On fabric, viability of bacterial isolates was inhibited for 14 days. GS also reduced recovery of viable MRSA, PA, and EC from Formica and stainless steel carriers treated with the product.
Conclusion: Our results demonstrate that GS has inhibitory activity and potential utility as part of an infection control process.
Key Words: Antimicrobial surfactant; methicillin-resistant *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Escherichia coli*; infection control.

According to the National Nosocomial Infections Surveillance system, nearly 60% of all *Staphylococcus aureus* infections are methicillin-resistant (MRSA), and 30% of *Pseudomonas aeruginosa* (PA) infections are fluoroquinolone-resistant.[1] Transmission of nosocomial infections, including multidrug-resistant organisms, is determined by a population of vulnerable individuals, a large cohort of colonized individuals, antimicrobial utilization, and adherence to infection control practices.[2,3]

The role of contaminated surfaces is a controversial area of infection control management. For some infections, contaminated surfaces or equipment moved between individuals is believed to be responsible. Even though every effort is made to reduce contamination on surfaces through proper practices and efficient cleaning reagents, the spread of infection continues. Among the microbes of greatest concern are *S aureus* and PA, particularly if these organisms have acquired antibiotic resistance.[1,4] Studies have reported that hospitalized persons with antibiotic-resistant *S aureus* have a greater probability of acquiring more symptomatic infections.[5] This increased risk is also associated with increases in hospital length of stay, morbidity, and mortality.[6,7]

Increased emphasis has been placed on infection control measures to reduce the growing number of antibiotic-resistant infections. A study of the transmission of vancomycin-resistant enterococci within a hospital found that 10.6% of the areas surveyed were contaminated via the hands of health care workers who contacted preexisting contaminated sites.[8] Such studies underscore the importance of handwashing and the prevention of bacterial contamination on environmental surfaces.

We were contracted to evaluate an antimicrobial surfactant (Goldshield 5 [GS hereinafter]; NBS Technology, Laurelton, NY) in our laboratory for its utility in reducing the bacterial burden from contaminated surfaces with continued protection, in contrast to current disinfectants. The core GS product is a quaternary ammonium salt that effectively inhibits the growth of mold, mildew, algae, and bacteria on a wide variety of materials, according to the manufacturer. GS is not a disinfectant, but rather is a surfactant that lends continued

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antimicrobial activity to already cleaned surfaces. The product is the first commercial application of technology developed at Emory University that has received 3 US patents (patent nos. US5,959,014, US6,221,944, and US6,632,805). The product is registered with the US Environmental Protection Agency (83075-1).

In this study, we tested the GS product on patient gowns at a 5% formulation with a 10% nonionic detergent, as would be formulated for use in the laundry, to determine its antimicrobial activity against patient isolates of MRSA, PA, and *Escherichia coli* (EC). We also tested a 1% formulation of the GS product containing a 10% nonionic detergent against these isolates using a carrier test procedure. Our data indicate that GS might be useful for long-term reduction of bacterial contamination on environmental surfaces.

MATERIALS AND METHODS

Bacterial isolates and culture conditions

Patient isolates obtained from hospitalized patients in 2005-2006 were frozen and stored at −70°C. Seven MRSA, 7 PA, and 8 EC isolates were grown on trypticase soy agar (TSA) plates or brain heart infusion plates for 18-24 hours at 35°C. Colonies from each isolate were used to inoculate 3 mL of Mueller-Hinton broth. Liquid cultures were grown overnight at 35°C. The optical density of organisms was measured with a Nanodrop 100 spectrophotometer (Nanodrop Technologies, Wilmington, DE) at a wavelength of 600 nm. Liquid cultures were diluted as appropriate.

Fabric test

GS and its formulations are marketed by HyGenesis (www.HyGenesis.com). Fabric testing was conducted according to American Association of Textile Chemists and Colorists protocol 100.[9] First, 2-inch circular swatches of fabric were cut from a patient gown consisting of a 50% cotton blend. All swatches were hand-washed in warm distilled water with nonionic detergent and allowed to air-dry completely. Test swatches were treated with 5% GS by thoroughly soaking the fabric in the product. The material was allowed to air-dry completely before inoculation. Four stacked swatches of treated and untreated fabric were inoculated with 4 mL of MRSA, PA, or EC at 0.5 McFarland. An additional control of untreated and uninfected material was established.

Sampling was conducted by placing each stack of 4 swatches in 100 mL of sterile saline solution and shaking for 1 minute. A sample aliquot was removed, and dilutions equivalent to 10^0, 10^1, and 10^2 were prepared. Then 100 μL from each dilution was plated onto TSA or brain heart infusion plates and incubated at 35°C for 18-24 hours. The resulting colonies were counted as appropriate.

Samples were collected at day 0, day 1, day 7, and day 14 without washing between samplings. Fabric was allowed to sit at room temperature (range, 21-24°C; humidity, 20%-40%) and was exposed to air for the period between samplings. The samples were diluted, plated, and incubated for 48 hours at 35°C.

Carrier test

Carrier tests were conducted according to a modified Association of Official Analytical Chemists use-dilution method.[10] Carriers of Formica (1 cm × 2.5 cm) and stainless steel (15-mm washers) were prepared by soaking for 30 minutes in 50% bleach and then rinsing several times with sterile deionized water. The carriers were stored in 70% ethanol until use. Treated carriers were submerged in GS for 15 minutes, allowed to air-dry, and then inoculated with 100 μL of 1×10^6 MRSA, PA, or EC in 10-μL droplets. The carriers were left at room temperature (range, 21-24°C) for 30 minutes and then placed into 10 mL of sterile phosphate buffered saline (pH 7.2) and vortexed for 2 minutes. Then 100 μL of this solution was plated onto TSA plates. Dilutions of 1:10 and 1:100 were also prepared from this solution and plated. Plates were incubated at 35°C for 24 hours, and colonies were enumerated.

RESULTS

Fabric tests

To determine the impact of GS on fabric, a patient gown was used for testing. The GS product was used at a 5% solution at the request of the distributor. This is the formulation that the company recommends for laundered materials under normal usage conditions. Patient gowns are more likely to be stored for several days to weeks after washing before being used. To simulate a more practical application, fabric was inoculated with bacteria and then kept at room temperature exposed to air for 14 days. Bacterial collection was performed immediately after inoculation and then at days 1, 7, and 14 after inoculation. The results are shown in Figure 1. In this experiment, the untreated material demonstrated a much slower decay in the number of organisms recovered compared with the treated material. Furthermore, PA and EC recovery from the treated material had a slower decay compared with MRSA isolates. These data demonstrate that GS is efficient in reducing the amount of viable organisms on contaminated fabric.