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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.

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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.

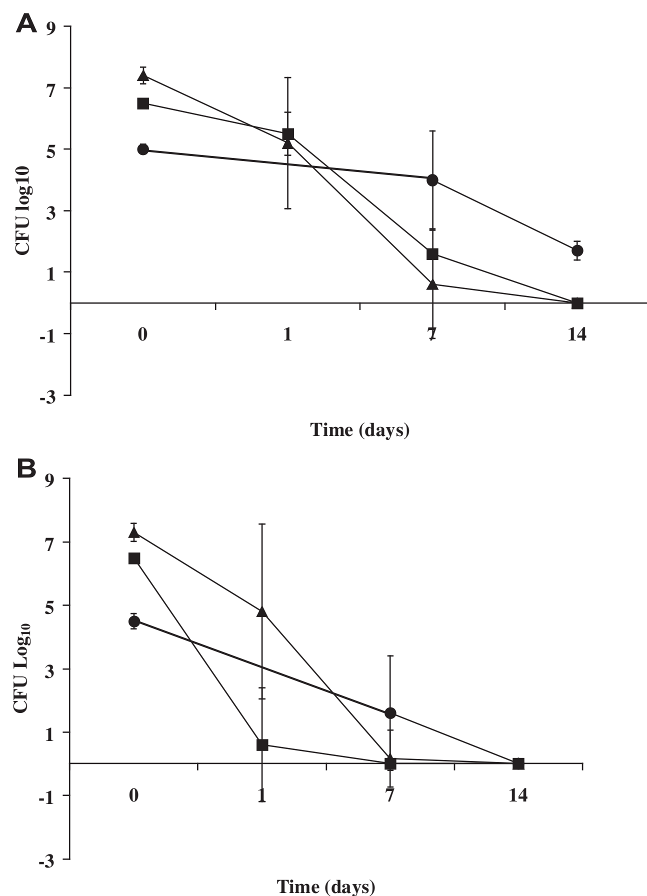

Fig 1. Fabric test. Patient isolates were applied to fabric treated with GS: MRSA (■), EC (●), and PA (▲). Viable recovery of bacteria from untreated (A) and treated (B) materials. Fabric was sampled every 7 days, with plated samples incubated at 35°C for 48 hours. Results are expressed as the mean for the number of isolates tested.

Carrier tests

The ability of the GS product to inhibit bacterial presence on Formica and stainless steel surfaces was evaluated using carrier testing.[10] Sterile carriers were prepared from Formica placards and stainless steel washers. For this experiment, GS was used at a 1% dilution containing a 10% nonionic detergent, as requested by the distributor. This is the formulation that the company currently markets for use on environmental surfaces. GS was found to reduce the viable bacterial burden of MRSA by 2.4 $\log_{10}$ on Formica and by 0.5 $\log_{10}$ on stainless steel, to reduce the bacterial burden of PA by 0.6 $\log_{10}$ and 0.8 $\log_{10}$, respectively, and to reduce the bacterial burden of EC by 0.9 $\log_{10}$ and 0.6 $\log_{10}$, respectively (Table 1).

Unlike disinfectants that must be reapplied continuously, the GS product purportedly exerts antimicrobial activity between applications. To detect residual activity on the carriers, bacteria were reapplied 4 days after the last sampling. Inhibitory activity was still observed, albeit with a reduction in effect. For MRSA contamination, the Formica carrier had a significant reduction of 2 $\log_{10}$, similar to the previous result. The reductions in viable MRSA on stainless steel carriers and of viable PA on both surfaces did not reach statistical significance, however. In contrast, reapplication of EC to these surfaces resulted in statistically significant reductions of 0.2 $\log_{10}$ on Formica and 0.5 $\log_{10}$ on stainless steel.

DISCUSSION

The increasing number of antibiotic-resistant organisms contributing to infection is a major concern. We evaluated a commercial product known as GS at a 5% solution for fabric application and at a 1% dilution for use as an antimicrobial surfactant at the behest of a commercial distributor. GS is a water-based organosilane that forms a silicon-nitrogen-carbon polymer as it dries after being applied to a surface. Organism survival on contaminated surfaces is prevented through mechanical disruption of microbial membranes, thereby inducing lysis of the organisms. This product has been tested for cytotoxicity using the International Organization for Standardization's agarose overlay method with L-929 mouse fibroblast cells by NAMSA Laboratories (Northwood, OH). Results of this unpublished testing found the product to have slight reactivity, defined as a 0-mm zone of cell lysis with some malformed or degenerated cells. This is considered grade 1 toxicity and falls below the standard of grade 2 as a cytotoxic agent.[11]

Previous unpublished in vitro studies have found that GS is capable of reducing the viable bacterial burden up to 99.9% on cotton material contaminated with American Type Culture Collection (ATCC) strains of *S aureus, Tricophyton mentagrophytes*, and *Aspergillus niger* (http://queenmar.net/resources/GTB-50 washes-project.pdf). Another unpublished in vitro study conducted by the University of Arizona, Department of Soil, Water and Environmental Science found long-term reduction of ATCC strains of MRSA and vancomycin-resistant enterococci from GS application using carrier test methodology, with a 99.99% reduction in viable bacteria over a 14-day evaluation. In both of those studies, application of the product differed from our methodology, however. The former study applied the product to the material using a commercial washing machine, whereas the latter applied the product using a spray bottle. In contrast, our method of application involved soaking all materials in the product. Another difference in our methodology is that we used clinical isolates

Table 1. Organisms recovered from Formica and stainless steel carriers treated with GS

Organism	Surface	Number of isolates	Log_{10} untreated ± SD	Log_{10} GS ± SD	Log_{10} reduction*,†
MRSA	Formica	7	5.6 ± 0.18	3.1 ± 0.14	2.4 ($P <.001$)
MRSA	Stainless	5	3 ± 0.38	2.6 ± 0.19	0.5 ($P = .03$)
PA	Formica	6	3.8 ± 0.7	3.2 ± 0.86	0.6 ($P = .05$)
PA	Stainless	6	3.8 ± 0.7	3 ± 0.75	0.8 ($P = .03$)
EC	Formica	8	6.1 ± 0.021	5.9 ± 2.11	0.9 ($P = .20$)
EC	Stainless	8	6.3 ± 0.28	5.8 ± 0.18	0.6 ($P <.001$)
Rechallenge‡					
MRSA	Formica	4	5.4 ± 0.13	3.5 ± 0.13	2 ($P <.001$)
MRSA	Stainless	5	3 ± 0.15	2.3 ± 1.32	0.8 ($P = .26$)
PA	Formica	4	4.2 ± 0.37	4 ± 0.55	0.2 ($P = .14$)
PA	Stainless	4	4 ± 0.56	3.8 ± 0.44	0.2 ($P = .71$)
EC	Formica	8	6.2 ± 0.12	6.1 ± 0.11	0.2 ($P = .01$)
EC	Stainless	8	6.3 ± 0.27	5.8 ± 0.47	0.5 ($P = .05$)

Results are expressed as mean for the number of isolates shown tested in duplicate.
*Reduction of bacterial growth reported as log_{10} after 30 minutes of contact with GS.
†*P* values calculated with the *t* test.
‡Rechallenge with bacteria was done at 4 days after initial sampling.

obtained from patients seen at our facility, whereas the other studies evaluated ATCC control strains.

Under our study conditions, GS produced considerable reductions in viable MRSA and, to a lesser degree, PA organisms. A possible explanation for this finding may lie in the biology of *Pseudomonas*. PA is known to have variable polysaccharide densities in its cell surface structure.[12] It may be that under our culture conditions, *Pseudomonas* became more resilient to mechanical disruption by the GS product. Another explanation might be the product's weaker affect on gram-negative viability, as demonstrated by the less significant reduction in EC isolates compared with MRSA isolates. The unpublished studies mentioned previously were conducted predominantly on gram-positive organisms. We believe that our study, although limited, provides further information on the utility of the GS product on gram-negative isolates.

Our carrier tests showed less pronounced inhibition of viable organisms compared with the fabric tests. The carrier test results demonstrate an affect of the surface being tested, with greater inhibition seen on the Formica carriers (and on the fabrics) compared with the stainless steel carriers. These findings suggest that the methodology used to evaluate the efficacy of antimicrobial surfactants is of great importance and must be considered carefully when analyzing data for claims of antimicrobial activity. Comparative analyses are needed to determine optimal application procedures and evaluation tools to maximize the bacterial inhibition on various surfaces.

Our results demonstrate that GS has inhibitory activity. The product's added benefit of an environmentally friendly composition may encourage its use in locations inappropriate for other, toxic agents. Based on our fabric and carrier tests, it appears that the inhibitory activity of GS depends on the organism and perhaps on the composition of the surface as well. Further evaluation is needed to adequately verify claims of product longevity on treated surfaces with a greater variety of gram-positive and gram-negative organisms.

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