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Major article

Persistence of nosocomial bacteria on 2 biocidal fabrics based on silver under conditions of high relative humidity

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Background: The survival of pathogenic microorganism on fabrics in the health care environment has a important role in nosocomial infections. The use of biocidal fabrics and surfaces could reduce the prevalence of the microorganisms in the hospital environment.
Methods: In this study, the persistence of nosocomial bacteria on 2 fabrics containing biocidal fibers (BF) in the long term following desiccation and subsequent storage was examined at 40% and 90% relative humidity (RH).
Results: Very few strains survived more than 7 days at 40% RH on fabrics containing 67% BF, and only strains of *Acinetobacter baumanii* and *Pseudomonas aeruginosa* survived on fabric containing 100% BF. None of the strains tested survived 14 days on the 2 fabrics, 67% or 100% BF, under these environmental conditions. In contrast, at higher RH (~90%), most of the strains tested showed prolonged survival on both fabrics, and all strains of *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *A baumannii* survived for more than 14 days; however, in a Petri dish, most of the microorganisms tested showed a higher survival even at 28 days. The gram-positive cocci and *A baumannii* were the most persistent bacteria on the Petri dish.
Conclusions: This study emphasizes the effect of RH on the survival of nosocomial bacteria on 2 commercially available fabrics containing biocide. Evidence of the clinical efficacy of these BF-containing fabrics is lacking.

Health care–associated infection (HCAI), also known as nosocomial or hospital-acquired infection, is recognized as the most frequent adverse event in health care. The European Centre for Disease Prevention and Control's pilot point prevalence survey of HCAI and antimicrobial use, involving 66 hospitals from 23 countries in Europe, noted that of 19,888 surveyed patients, 7.1% had a nosocomial infection and 34.6% were receiving at least 1 antimicrobial agent. Infection prevalence was highest in intensive care units, at 28.1%.[1]

Although the major source of HCAI is probably patients' endogenous flora, an estimated 20%-40% of cases may be attributed to cross-infection via the hands of health care personnel.[2] Contamination of the hands of these workers occurs mainly through direct contact with other patients, but also may occur through contact with textiles and other surfaces in the hospital environment.[3] Numerous outbreaks have been attributed to environmental sources and have been brought under control after removal of the source.[4,5] Furthermore, the use of disinfectants and strict compliance with infection control practices are vital to interrupt transmission, but this has not been sufficient to reduce HCAI rates in most cases.[6] In general, resistance to commonly used disinfectants might not play an important role in nosocomial infections, but other factors, such as lack of compliance with manufacturers' recommendations, insufficient contact times, or the presence of organic matter, may be critical.

Although contamination of textile and nontextile surfaces plays a limited role in the spread of nosocomial infection, numerous investigations have been directed toward the development of materials for clinical use to reduce the persistence of pathogenic microorganisms.[7] Many of these studies have focused on the use of biocides, particularly the development of coatings that release antimicrobials or kill microorganisms on contact.[8] Another strategy has been the development of materials that limit bacterial

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adhesion.[7] In some studies, the use of copper surfaces in the hospital environment has been associated with a significant reduction in bacterial burden on common surfaces.[9] A recent study reported a significantly lower prevalence of HCAI in patients assigned to rooms with copper surfaces (3.4%) compared with patients in traditional rooms (8.1%).[10]

The antimicrobial activity of surface coatings containing silver and silica[2] and of silver-based antimicrobial fabrics[11] has been investigated as well. Although in vitro studies have demonstrated the antimicrobial effectiveness of most of these materials under controlled environmental conditions, studies of the effectiveness of these materials in reducing HCAI have often provided unclear results. Numerous microbiological and environmental factors can affect the antimicrobial properties of these materials. Sometimes, depending on the use to which the fabrics are designed (eg, bandages, towels, cloth wipes, pajamas), conditions of high relative humidity (RH) in the hospital environment can be expected.

The present study was undertaken to determine the influence of high RH on the long-term persistence of nosocomial microorganisms on the biocidal activity of 2 silver-based antimicrobial fabrics. Several bacterial strains isolated from hospitalized patients with nosocomial infections, including methicillin-resistant *Staphylococcus aureus* and *Acinetobacter* strains, were selected for study as representative of the most common bacteria seen in HCAI.

MATERIALS AND METHODS

Organisms and inoculum preparations

Thirty-eight strains of nosocomial bacteria isolated from patients were procured from the microbiology laboratory of the University Hospital of Málaga. Gram-negative bacteria were maintained on tryptic soy agar (TSA; Panreac) slants and subcultured monthly. Columbia agar with sheep's blood (Oxoid) was used for gram-positive bacteria.

To prepare a working culture, bacterial strains were grown in 10 mL of tryptic soy broth (TSB; Panreac) at 37°C for 22 hours, harvested by centrifugation at 9,000 × *g* for 5 min at 4°C, and rinsed twice with 0.9% (w/v) saline solution (SS). The rinsed bacteria were then suspended in SS and adjusted to an optical density of 0.1 ± 0.01 at 630 nm. The fresh cell suspension was maintained at 4°C and used as the standard bacterial suspension (SBS) in experiments within 1 hour.

Fabrics test

The test biocidal fabrics (BFs) were Bioactive-treated polyester/cotton (80/20) and Bioactive-treated 100% polyester, both marketed by Interasa, containing 67% and 100% bioactive polyester fibers in the finished fabric, respectively. (The silver ion concentration on the bioactive silver is approximately 180 ppm, according to the manufacturer.) Untreated polyester/cotton (80/20) fabric served as a control fabric (CF).

Desiccation survival assay

For this assay, 100 μL of SBS was sprayed onto dry sterile Petri dishes in a safety cabinet, and the dishes were air-dried in a chamber for 4 h at 22 ± 2°C and 40% ± 5% RH. The number of bacteria before desiccation (time 0) was determined by plate count. For testing of long-term persistence, dried dishes were placed in an incubator at a temperature and RH similar to the mean values observed in the environment hospital (approximately 22°C and 40% RH). The number of surviving bacteria at 24 hours and 1, 2, 3, and 4 weeks was determined by adding 15 mL of sterile SS and 2 g of 3-mm glass beads. The Petri dishes were shaken for 10 min at room temperature on an orbital shaking incubator, after which 1 mL of bacterial suspension was serially diluted (1:10) and plated in triplicate on TSA. Colony counts were recorded after a 24-hour incubation at 37°C, and are expressed as the percentage of survival bacteria at each time point. The number of bacteria before desiccation (time 0) was considered to be 100%.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of silver nitrate was investigated for all strains in liquid medium on 96-well microtiter plates, following a previously described protocol.[12] In brief, 50 μL of 2× TSB inoculated with a bacterial suspension containing approximately 5×10^7 CFU/mL was mixed with an equal volume of silver nitrate to give final silver concentrations of 1, 2, 4, 8, 16, 32, and 64 mg/L. After a 24-hour incubation at 37°C, the free silver was quenched by adding 5 g/L $Na_2S_2O_3$. Then a series of 1:10 dilutions of each microtiter plate (n = 3) was made in SS, plated on TSA, and incubated for 24 hour at 37°C to determine the MIC.

Evaluation of antibacterial activity of the fabrics

This evaluation was performed using a slightly modified method of the American Association of Textile Chemists and Colorists.[13] First, four stacked 2-inch circular swatches of test fabric (BF67 or BF100, with 67% and 100% bioactive fibers, respectively) or CF were placed in a glass Petri dish and autoclaved for 60 min. The swatches were then inoculated with 4 mL of SBS, ensuring even distribution, and left to dry in the open Petri dish in a safety cabinet for 45 min. The stacks of inoculated and dried swatches of each test fabric (inoculated and noninoculated) were placed in desiccators maintained at 22°C and containing a dish with demineralized water or saturated K_2CO_3, to obtain ~90% or 40% RH respectively. During all experiments, both temperature and RH were monitored continuously using a data logger (HI9564; Hanna Instruments). Sampling was conducted at 0 h (immediately after drying of the inocula), 24 hours, 1 week, and 2 weeks by homogenizing each stack in 100 mL of sterile SS in a laboratory blender (Stomacher 400; Seward) for 2 min. Aliquots (900 μL) were then plated in the first column of a 48-well plate containing 100 μL of 10× TSB per well, and decimal dilutions were performed into the subsequent columns, each containing 900 μL of TSB per well, using a multichannel pipette with new pipette tips for each dilution. The plates were covered with a lid and then incubated for 24 hours at 37°C. In each plate, six different strains were tested, and the highest dilution in which growth occurred (HD) was used to estimate bacterial concentration, as described previously.[14]

Statistical analysis

The antimicrobial activity of each test fabric was calculated as the percentage of viable bacteria from the mean HD for fabric containing bioactive fibers (BF67 or BF100) and the CF. Survival was calculated for each strain at 1, 7, and 14 days after desiccation, and the HD of bacteria at time 0 (after drying for 45 minutes) was considered to be 100%. All trials were performed in triplicate and were repeated for each strain on 3 different days. The SD of the replicas (repeatability SD) was then calculated, and Dunnett's one-sided many-to-one test was performed to compare the HD of the BFs and CF at different time points for each strain. Statistical analyses was performed with SPSS software Ver.13.0 (SPSS Inc, Chicago, IL).