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Title: In vitro and in vivo testing of microbe growth on antimicrobial nursing scrubs

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Abstract

Background/Significance: 5–10% of hospitalized patients develop a hospital-acquired infection (HAI). Scrubs are a potential vector of HAIs.

Purpose: To compare the antimicrobial characteristics of scrubs with and without an antimicrobial fabric coating, as tested in the laboratory (in vitro) and hospital (in vivo) environments.

Methodology/Design: Two protocols were conducted to address the purpose. The in vitro protocol was a laboratory study that involved observing the presence of microbe growth after inoculating coated and uncoated scrub fabric swatches with *S. aureus* and then processing them in moist and dry environments. The in vivo protocol was a clinical trial that measured microbe growth on coated and uncoated scrubs prior to and following nursing staff completing a 12 h shift on an acute care unit, as measured by colony forming units (CFUs).

Results: For high humidity environments, the in vitro study indicated that swatches treated with an antimicrobial coating exhibited minimal microbe growth while untreated swatches exhibited significant microbe growth. For low humidity environments, coated and uncoated swatches were all judged to exhibit minimal microbe growth. In the in vivo study, the CFUs increased on scrubs worn by nurses over a 12 h shift with no significant difference in CFUs for coated and uncoated scrubs.

Discussion: For bacteria in a warm and moist environment, the antimicrobial coating was found to be important for inhibiting growth. For bacteria in a warm and dry environment, both coated and uncoated fabrics performed similarly as measured at 24 h, with minimal bacterial growth observed. In a hospital environment, microbe growth was observed but no significant difference was detected when comparing coated and uncoated scrubs. This may have been due to the short time between exposure and culturing the scrubs for analysis immediately at the end of shift, not allowing for enough time to kill or inhibit growth. Contact time between the bacteria and scrub fabric (coated or uncoated) in the in vivo study more directly correlated with the 0 h observations for the in vitro study, suggesting that the ineffectiveness of the treated scrubs in the clinical results may be due in part to short residence times before collection.

Background

Nosocomial or healthcare-associated infections (HAI) are infections acquired by patients during their admission to a health care facility[1]. The CDC estimates that 1 in 31 hospitalized patients and 1 in 43 nursing home residents at any time has an HAI[2]. Previous researchers have suggested that an increasing number of HAIs are caused by microbes that are resistant to antibiotics that have previously been effective in treating HAIs[3]. For example, methicillin-resistant Staphylococcus aureus (MRSA) is a microbe commonly implicated in HAIs, with the first case of MRSA being documented in 1968, only 8 years following the clinical introduction of methicillin to treat S. aureus infections[4].

In 2019, 700,000 people died worldwide due to microbes that are resistant to antibiotics and by 2050 the figure is estimated to rise to 20 million, with an estimated cumulative cost of $2.9 trillion[5]. Antibiotic resistant HAIs are also associated with prolonged length of hospital stay and higher rates of in-hospital mortality[6]. The evolution of these antibiotic resistant microbes that result in HAIs are the result of widespread use, overuse, and misuse of antibiotics during the last 80 years[7]. On any given day, about half of hospitalized patients and 1 in 12 nursing home residents are receiving an antibiotic medication[2].

To compound the problem of increasing numbers and strains of antibiotic resistant microbes, several pharmaceutical companies have abandoned antibiotic research and the development of new antibiotics[8]. Although the prospect of fewer effective antibiotics to treat HAIs is discouraging, several scientific advancements may have the potential to reduce HAIs that are not based on antibiotic medications.

One approach to reducing HAIs is to break the chain of infection by reducing the mode of transmission of microbes on the garments of healthcare providers. Findings suggest that

provider attire is a source of pathogenic bacterial transmission in health care settings[9]. A number of antimicrobial materials have been used to coat fabrics including metals, metal oxide nanoparticles, and functionalized silica nanoparticles in attempts to produce antimicrobial (antibacterial and antifungal) fabrics[10, 11]. These fabrics can then be used to manufacture garments (commonly referred to as scrubs) for health care providers to wear while in contact with patients.

Fabrics coated with these materials in controlled (in vitro) environments have demonstrated highly effective antimicrobial properties[12-14]. Based on this evidence, garment manufacturers have coated scrub uniforms with these materials and marketed them as antimicrobial[15, 16] making the healthcare scrub market into a $10 billion business annually[17]. However, these promising antimicrobial characteristics have not been replicated in studies where coated scrubs are worn by healthcare staff in the clinical (in vivo) environments[18-20].

More recently an extensive review of the related literature concluded that antimicrobial coatings applied to bed and bath linens and patient clothing showed some promise in reducing microbes on these surfaces, although no definitive efficacy was detected in reducing microbes when applied to healthcare workers' uniforms[21]. A gap in the literature exists when explaining why antimicrobial coatings on fabrics are effective in in vitro (laboratory) environments but are not effective in in vivo (hospital) environments, which we address in this study.

Purpose: To compare the effectiveness of antimicrobial scrubs in the laboratory (in vitro) versus the hospital (in vivo) environment. This purpose was addressed through two trials evaluating the following two hypotheses:

RH1: Fabric swatches of scrub fabric treated with MicroBlock™ antimicrobial coating will exhibit minimal microbe growth when compared with untreated fabric swatches within both moist and dry in vitro environments.

RH2: Scrubs worn by nursing staff on an acute care unit within a hospital (in vivo) environment will exhibit lower CFUs after a 12 h shift when treated with MicroBlock™ antimicrobial coating than compared with untreated scrubs.

Methods

In Vitro Protocol

Samples of fabric swatches were obtained from two pairs of scrub pants (Cherokee® brand, Van Nuys, California; 78% polyester/20% rayon/2% spandex blend). These two pairs of scrub pants were selected randomly from the stock of scrub sets that were prepared for the clinical study, such that the in vitro and in vivo fabrics were the same. One of these scrub pants had been exposed to the MicroBlock™ antimicrobial coating using a proprietary fabric coating process. MicroBlock™ is silver-based, which is a natural antimicrobial. The other scrub pants were processed in the same way but without the coating compound.

The antimicrobial activity of the coated scrubs was first verified using the American Association of Textile Chemists and Colorists Test Method 100 (AATCC-TM100)[22], which is an industry standard test protocol for quantitatively evaluating the antimicrobial activity of treated textiles (see Figure 1). Briefly, fabric swatches were cut from one of the unused scrubs

sets, inoculated (*staphylococcus aureus* and *klebsiella pneumoniae* were both tested) and then incubated for 24 h at 37 °C in a sealed container. The bacterial challenges were then eluted from the swatches, plated in a series of dilutions, incubated for 24 h at 37 °C, and enumerated. Uncoated scrubs were used as a control for the coated scrubs.

Next, to evaluate antimicrobial activity in a way that is comparable to our clinical study, we developed the following quantitative test protocol. Twenty 2.5 cm x 2.5 cm swatches were obtained from each of these scrub pants. The swatches that were exposed to the MicroBlock™ coating were labeled as "coated swatches" while the swatches not exposed to this coating were labeled as "control swatches." Following this grouping, the swatches were all inoculated with *Staphylococcus aureus* (ATCC #6538). The *S. aureus* inoculate was diluted from an overnight culture by a factor of 10^6 before being used to inoculate all fabric swatches. This dilution resulted in an average of 250 potential CFU's delivered to each swatch, where the CFU count was estimated using the serial dilution method. Each of the swatches was inoculated such that the fabric was fully saturated in a sterile petri dish, but with no runoff or excess liquid.

Ten coated swatches and ten uncoated swatches were incubated at 37 °C within a high humidity environment for 24 h. For high-humidity incubation, the covered petri dishes were sealed with parafilm and then placed in sealed containers along with wet paper towels, all of which was placed in the incubator. Those swatches were found to be wet after the incubation period. Another ten coated swatches and ten uncoated swatches were incubated at 37 °C in a low humidity environment for 24 h, resulting in complete drying of those swatches. For low-humidity incubation, the covered petri dishes were sealed with parafilm and then placed in sealed containers but without paper towels, all of which was placed in the incubator. Those swatches were found to be dry after the incubation period.

Following this initial incubation period all fabric samples were transferred to sterile plates containing agar. The agar plates were made using a standard Luria Broth (LB) recipe with added agar that was autoclaved and poured aseptically into sterile petri dishes to make up the medium used to assess bacterial survival and growth. Transfer of bacteria to these plates involved placing the fabric swatch in contact with the agar surface and then gently applying pressure and smoothing the fabric on the agar (stamping) for 30 seconds. The swatch was then reversed, and the opposite side of the swatch was placed on a different site on the agar plate and stamped for 30 seconds. The swatch was then removed from the plate and disposed of in a biohazard receptacle. All plates were then covered and incubated at 37 °C for 24 h.

After incubation, the number of CFUs on each plate was assessed separately by two evaluators. Initially, the number of colonies were planned to be counted on each plate, but following initial observations of the plates the decision was made to evaluate CFUs qualitatively by each evaluator, independently classifying the plate as "microbe growth present" or "minimal microbe growth present". This decision was made due to the prevalence of plates without countable CFUs, both in the sense of no CFUs and too many to count (see Figure 2).

In Vivo Protocol

Design: A double-blind randomized clinical trial measured the CFUs on treated and untreated scrubs worn by nursing staff during a 12 h shift on a hospital unit. These scrubs were worn by a sample of 26 nursing staff who had a patient care assignment on one of two clinical units within an acute care hospital. These participants wore a different set of scrubs on two consecutive 12 h shifts. Each scrub set consisted of Cherokee® brand workwear scrubs pants and a short sleeve scrub top that were a 78% polyester/20% rayon/2% spandex blend. The long sleeve t-shirt

included in the scrub set was 65% poly/35% cotton and was worn under the scrub top. One of the sets was treated with MicroBlock™ antimicrobial coating and the other set was processed in the same way but without the coating compound. This was the same as the in vitro study.

Sampling of the scrubs was done to determine the number of CFUs on the scrubs at the beginning (baseline) and end (post-shift) of the same 12 h shift. These samples were collected on RODAC plates and then transported and processed following the 12 h shift by the same microbiology laboratory where the in vitro protocol was conducted. The number of CFUs were documented at baseline and post-shift for each scrub type. This resulted in a two-by-two repeated measures design in which each subject had CFUs sampled at 2 times (baseline & post-shift) under two experimental conditions (scrubs with or without MicroBlock™). The nursing staff wearing the scrubs, and the research assistant providing the scrub sets, collecting them, and processing the samples were all blind to which scrub set contained the MicroBlock™ coating. In addition, potential covariates were collected from each subject including the number of patients they were assigned to for direct care, the number of patients on the unit, and the number of patients in any type of isolation.

A sample of 26 nursing staff who provided direct patient care and were scheduled to work two consecutive 12 h shifts on one of two progressive/intermediate acute care medical surgical units within a level one trauma hospital were recruited by fliers posted in break rooms and locker/rest rooms. Potential subjects were told their participation would be voluntary, and their level of participation and individual data would NOT be shared with their employer. A sample size of 26 nursing staff measured at two times (baseline and post-shift) under two experimental conditions (scrubs with or without MicroBlock™) yielded 0.80 statistical power, when $\alpha = 0.05$ to detect a 0.3 effect size difference of scrub set type on the outcome variable of

CFU[23]. Individuals eligible for the study were nursing staff aged 18 years and older, who had direct patient contact, who were scheduled to work two consecutive 12 h shifts on the same hospital unit during the trial. Individuals were excluded from the trial if their work role during the study involved any activities which would limit their patient care contact, including administrative, research, or committee activities.

Data Collection: Individuals who responded to the recruitment flier signed an informed consent form prior to any data collection (IRB#300325-UT) and completed a background questionnaire. The research staff met subjects on their clinical unit just prior to the start of their shift and provided them with a new scrub set including a scrub top, scrub pants and a long sleeve t-shirt. Subjects were asked not to wear any additional personal clothing over their scrubs except for protective garments required to complete their job (gloves, isolation gown, mask, footies, etc.).

Once prepared, each scrub set was packaged in sealed plastic bags (identified as only "type 1" or "type 2") and then delivered to the clinical research staff. Only chemistry laboratory staff knew which scrub sets were and were not treated, and these individuals were not involved in data collection, processing of the samples, or data analysis.

Immediately following each participant donning the scrub set on the acute care units, the research assistant obtained samples from six sites on the scrubs by lightly pressing a sterile RODAC plate against the collection site for 15 seconds each (sleeves ×2, midriff ×2, pants cuffs ×2). This procedure has been previously developed to assess antimicrobial characteristics of clothing worn by health care workers [24, 25]. Each RODAC plate was then sealed, labeled, and transported to the microbiology lab for processing.

At the end of the 12 h shift the research assistant again obtained samples from the same six sites. These plates were also sealed, labeled, and transported to the microbiology laboratory

for processing. All plates were incubated for 24 h at 37 °C. The following day, the number of CFUs on each plate was counted by a trained laboratory assistant who was also blind to the type of coating on the scrub from which each sample was obtained.

Results

The antimicrobial activity of the coated scrubs was established using the AATCC-TM100 test protocol. The results clearly showed that the coatings applied to the scrubs were effective, with no CFUs found on the MicroBlock™ treated scrubs fabric (see Fig. 1). In contrast, the untreated scrubs control showed no significant antimicrobial activity.

RH1 was addressed by two trained evaluators separately, who visually inspected the plates and qualitatively classified each plate as "microbe growth present" or "minimal microbe growth present". Figure 2 presents a photograph of the 20 plates from swatches that were incubated at 37 °C and high humidity before stamping. The two evaluators unanimously agreed that all ten plates exposed to the coated swatches were classified as "minimal microbe growth present" while all ten plates exposed to the control swatches were classified as "microbe growth present."

The photograph in figure 3 displays the 20 plates from swatches that were incubated at 37 °C in low humidity (resulting in their drying) before stamping. The two evaluators unanimously agreed that all 20 plates in figure 3 were classified as "minimal microbe growth present."

To address RH2, data were transcribed from the data collection forms that recorded CFU counts to an SPSS spread sheet. Descriptive statistics were calculated to describe the demographic characteristics of the samples reported by the participants on the background questionnaire. To test study hypothesis two, separate repeated measures ANOVAs (R-ANOVA)

were calculated to compare CFUs measured at each of the six sites for treated and untreated scrub samples collected at the start (T1) and end (T2) of the shift. Significant main effects were further explored through calculating Tukey's post hoc comparisons to determine differences within and between the groups over the 2 data collection time points at each of the data collection sites.

Of the 26 participants, 80% were female, with a mean age of 32.65 ± 8.36 years, with 7.46 ± 5.41 years of nursing experience and working an average of 41.19 ± 9.14 hours per week. Table 1 indicates that the sample of 26 nurses were assigned a similar number of patients while wearing the coated (4.92 ± 3.50) and uncoated (4.69 ± 2.71) scrub sets. The unit census and the number of patients in any type of protective isolation were also similar when the participants wore the coated and uncoated scrubs.

Table 2 presents the results of the R-ANOVAs analyses that compared the CFUs measured at each of the six sites comparing coated and uncoated scrubs collected at the start and end of the shift. For analysis involving the right sleeve, left sleeve, right midriff and left midriff the findings were similar. For each of these sites there was a significant ($p<.05$) time effect with post hoc comparisons indicating that the CFU counts increased between the start and end of shift for all of these sites for coated AND uncoated scrubs, with no group or interaction effect of any of these sites. The CFU counts on the right and left cuffs of the scrubs were not significantly different between the scrub coating groups at the start or end of shift and the CFU count did not change within the scrub coating groups at these two cuff sites between the start and end of shift.

Discussion

The results of the in vitro study only partially support RH1. The qualitative interpretation of figure 2 supports RH1. This figure indicates that plates obtained from coated swatches that were initially incubated at 37 °C and high humidity were consistently rated as "minimal microbe growth." The plates obtained from uncoated control swatches under these same conditions were consistently rated as "microbe growth present." This figure supports the hypothesis that swatches that were treated with the MicroBlock™ antimicrobial coating will inhibit bacterial growth within a moist in vitro environment.

By contrast, the qualitative interpretation of figure 3 does not fully support RH1. This figure indicates that plates obtained from all 20 swatches (coated and uncoated) that were initially incubated at 37 °C and low humidity were rated as "minimal microbe growth". This figure supports the hypothesis that treated swatches will inhibit bacterial growth within a dry in vitro environment, but it does not support the hypothesis that untreated swatches will not inhibit bacterial growth within a dry in vitro environment.

These findings indicate that when the environment was optimal for microbe growth (high humidity), the MicroBlock™ antimicrobial coating inhibited microbe growth and/or survival. On the other hand, these findings also indicate that when the environment was not optimal for microbe growth (low humidity), microbe growth was not observed independent of whether or not the fabric had an antimicrobial coating. There are two possible explanations for why microbe growth was not observed in the case of dry uncoated fabrics: (i) the microbes perished due to the dry environment, and (ii) the microbes survived but did not grow into observable colonies in the dry environment. There are three possible explanations for why microbe growth was not observed in the case of dry coated fabrics: (i) the microbes perished due to the dry environment,

(ii) the microbes survived but did not grow into observable colonies in the dry environment, and (iii) the MicroBlock™ coating inhibited microbe growth and/or survival.

The results of the study conducted in the in vivo environment did not support RH2. Table 2 indicates that both control and treated scrubs exhibited significant increases in CFUs over the 12 h shift at the four upper body sampling areas (sleeves and midriff, bilaterally) but no significant increase in CFUs on the lower body sampling areas (pant cuffs, bilaterally). There was no significant difference when comparing CFUs on treated and untreated scrubs.

The CFUs on the treated scrubs appeared to be lower at 5 of the 6 sites sampled at the start of shift, however, when compared to control scrubs at the start of shift. A second observation, although not statistically significant, was the differences between control scrubs and treated scrubs over the shift at 4 of the 6 areas sampled. The increase in CFUs was observed to be higher on treated scrubs (8% to 317%) compared to control scrubs (-10% to 288%).

Although not consistent with the original hypotheses, there is support for these findings from the literature. Previous investigators have reported that fabrics coated with antimicrobial materials significantly inhibit microbe growth within controlled in vitro environments[12-14]. However, these coatings have not been found to significantly inhibit microbe growth in a hospital environment[18-20, 24]. The results of this study provide some explanation for this inconsistency. First, our in vitro study indicates the importance of humidity or water in the environment in activating the antimicrobial coating. In the presence of water, the silver in the MicroBlock™ likely ionized at a greater rate and provided a mechanism to transport the ionized silver to the microbes. Since the number of CFUs were not statistically different for the treated and untreated scrubs following a 12 h shift, one potential explanation is that the hospital environment is too dry to activate the MicroBlock™ coating. This explanation is consistent with

the fact that hospital environments in general are kept at low levels of humidity to minimize microbes in the environment[26-28].

Second, our in vitro studies showed that bacteria did not survive on coated fabrics in both wet and dry conditions, but this was not consistent with the results of our clinical study. It is reasonable to assume, however, that there is some kinetic component to bacterial death, meaning a certain period of time may be necessary to kill the bacteria. Therefore, one might explain the difference between the laboratory and clinical results by considering that the observed bacteria may have inoculated the scrubs in the clinical setting within this time period. This was not accounted for in the clinical study, meaning it is possible that not enough time had elapsed to kill the bacteria on the scrubs before being collected immediately at the end of shift (see Figure 4).

Third, the analysis of those clinical results showed no statistical difference between coated and uncoated scrubs. Given that bacterial death was also observed on dry uncoated fabric in the laboratory study, it is possible that the rate of bacterial death on the dry scrubs may have been similar enough for coated and uncoated fabrics to yield statistically similar results. In this case, one might conclude that if scrubs are kept dry then whether or not they have an antimicrobial coating may not be important. One might also conclude, however, that if scrubs are not kept dry then it may indeed be important for an antimicrobial coating to be present.

The results of the study must be interpreted cautiously due to these issues, which may have impacted the outcomes of the in vitro and in vivo experiments. Future investigators may design in vivo studies testing the efficacy of antimicrobial coatings on scrubs while more tightly controlling for humidity in the environment. Further, the time required for antimicrobial action is not currently known. Testing scrubs immediately at the end of a hospital shift may not provide the time required for antimicrobial action to result in a measurable in inhibition effect.

Conclusion

Although antimicrobial coatings (including MicroBlock™), have been shown to inhibit microbe growth in controlled in vitro environments, there is limited evidence that these scrub coatings significantly inhibit microbe growth within the hospital environment. Our results indicate that humidity and time may be important variables that may affect the efficacy of antimicrobial coated fabrics. Future studies need to explore the effect of varying environmental characteristics (humidity, temperature, exposure to microbes, etc.) on the ability of these coatings to inhibit microbe growth as well as the temporal component of the antimicrobial function of these fabrics.

Table 1: Demographic description of the in vivo sample.

Characteristics of the Unit	Scrub Coating	Mean + SD	Statistical Comparison
Number of patients assigned per nurse	Without MicroBlock™	4.69 ± 2.71	t=.27, p=.23
	With MicroBlock™	4.92 ± 3.50	
Patient census of the unit	Without MicroBlock™	18.12 ± 6.18	t=.43, p=.67
	With MicroBlock™	17.46 ± 4.70	
Number of patients on the unit in isolation	Without MicroBlock™	4.04 ± 2.03	t=.37, p=.72
	With MicroBlock™	3.85 ± 2.13	

Table 2: Comparison of CFU at 6 sites before and following 12 h hour shift between control scrubs and scrubs treated with MicroBlock™.

Scrub Coating	Time	Rt Sleeve	Lt Sleeve	Rt Midriff	Lt Midriff	Rt Cuff	Lt Cuff
Without MicroBlock™	Start of Shift	48.55+40.56	49.04+52.66	48.89+55.52*	28.69+29.54	41.62+41.48	37.85+43.81
	End of Shift	118.35+69.96*	120.54+79.19*	103.19+15.98	111.50+84.88*	37.35+26.25	52.808+6.38
Percentage Change from Start of Shift		144%	146%	111%	289%	-10%	40%
With MicroBlock™	Start of Shift	39.08+36.38	31.12+40.08	37.46+43.17	32.12+30.40	29.92+35.77	30.19+30.25
	End of Shift	122.89+91.81*	129.73+125.13*	99.96+15.98*	92.92+83.25*	35.23+36.29	32.58+14.07
Percentage Change from Start of Shift		214%	317%	167%	189%	18%	8%
Statistical Comparison		G: F=.32, p=.86 T: F=48.9, p=.00 GxT: F=.41, p=.53	G: F=.11, p=.75 T: F=44.03, p=.00 GxT: F=1.12, p=.29	G: F=.29, p=.59 T: F=22.76, p=.00 GxT: F=.11, p=.74	G: F=.35, p=.56 T: F=37.66, p=.00 GxT: F=.88, p=.35	G: F=1.10, p=.30 T: F=.01, p=.92 GxT: F=.80, p=.38	G: F=2.75, p=.10 T: F=1.49, p=.23 GxT: F=.79, p=.38

Note: * indicates a significant change ($p<.05$) over time within the scrub coating.

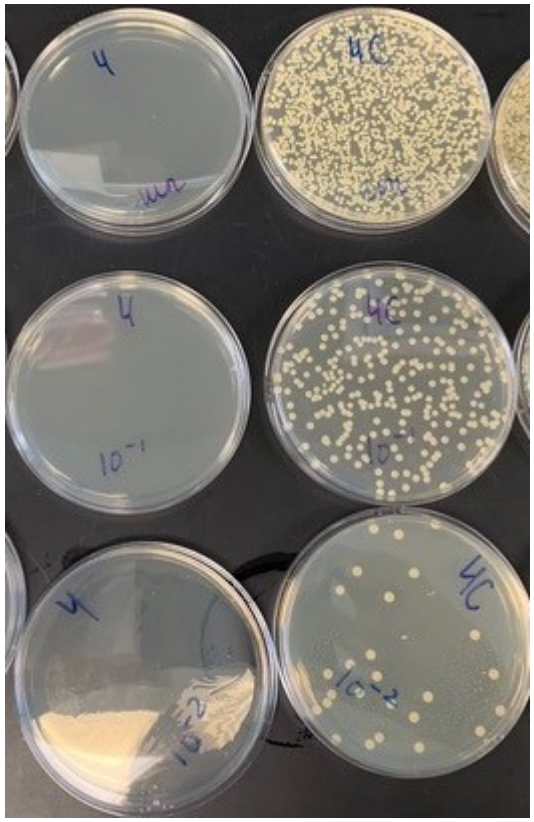

Figure 1: Antimicrobial activity of coated scrubs evaluated using the AATCC-TM100 test protocol. (left column) Scrubs coated with MicroBlock™ and (right column) uncoated scrubs. Scrubs were rinsed and dried before use. Results for *S. aureus* challenge are shown. Top row corresponds to 100 µL of 100 mL eluted inoculum, middle is diluted 10x, bottom is diluted 100x.

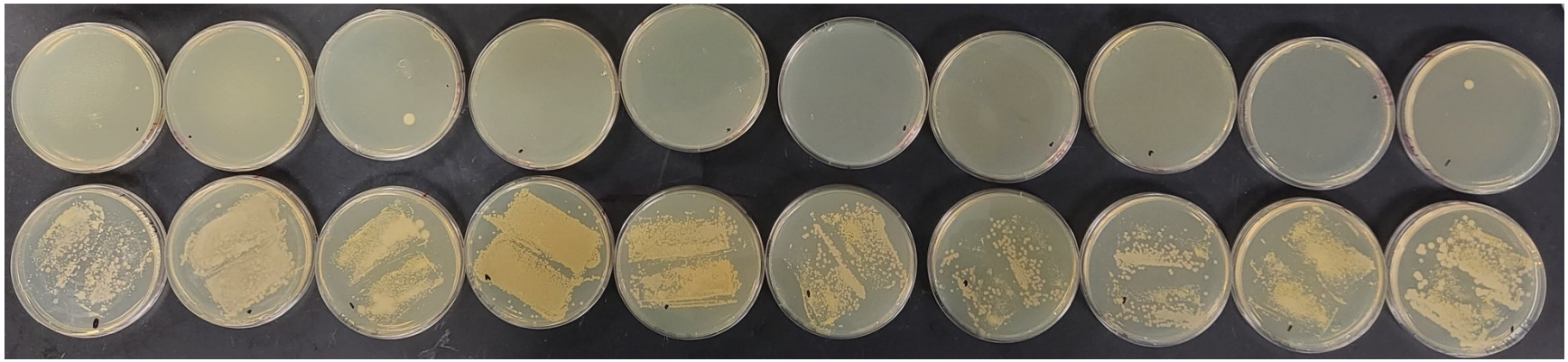

Figure 2: Plates obtained from fabric swatches that were incubated at 37 °C with high humidity, which kept the fabric swatches wet. The plates in the top row are from coated swatches and the bottom row are from uncoated control swatches. All swatches were inoculated with *S. aureus*.

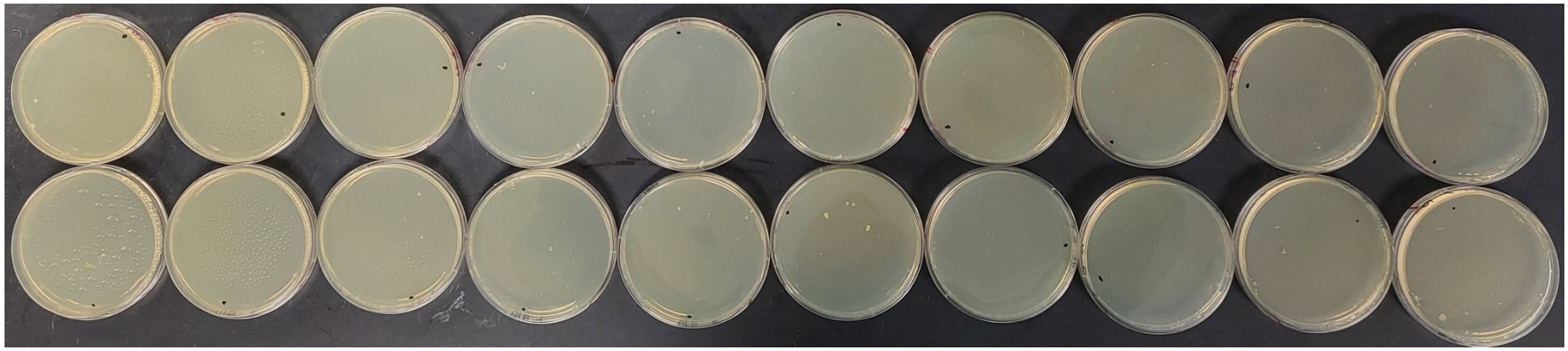

Figure 3: Plates obtained from fabric swatches that were incubated at 37 °C with low humidity, which dried the fabric swatches. The plates in the top row are from coated swatches and the bottom row are from uncoated control swatches. All swatches were inoculated with *S. aureus*.

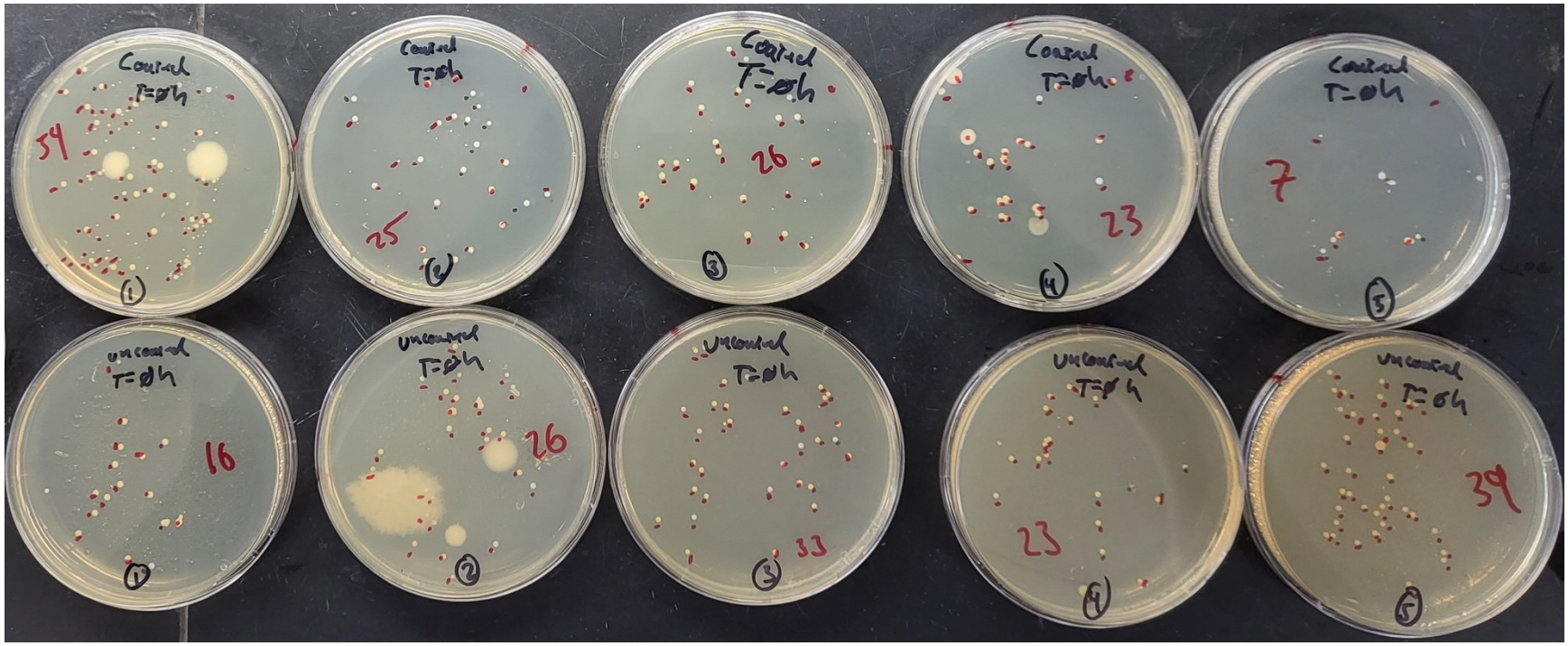

Figure 4: Plates obtained from coated and uncoated fabrics immediately after the swatches were inoculated with *S. aureus*. The plates in the top row are from coated swatches and the bottom row are from uncoated control swatches. Only *S. aureus* was counted.

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