

## Research Article

### The *In Vitro* Effectiveness and Toxicity of a Quaternary Ammonium Compound as an Antimicrobial Finish on Denim

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#### Abstract

Antimicrobial textures have been used in the textile market for decades. Coated textile fabrics possess a wide range of applications in the defense and civilian sectors. In hospitals, these coated fibers are used to mitigate cross contamination from microbes. Various types of antimicrobial finishes are currently available including oxidizing agents like aldehydes and halogens, quaternary ammonium compounds, metallic compounds like cadmium, silver, and natural antimicrobial agents like chitosan and neem. Each group of these antimicrobial finishes has both different properties and different modes of actions against microbes. However, the current body of research regarding the toxicity of these chemicals on humans is limited. This *in vitro* study investigated the toxic effects of these antimicrobial QAC quaternary ammonium compound finish on human epidermis. The Hs733Sk human skin cell line was used to evaluate toxicity with measures of cell viability and  $IC_{50}$ . Assessment of toxicity is important, as it is a limiting factor in the amount of antimicrobial finish that can be coated on the denim fabric even if greater concentrations are required to mitigate the growth of microbes. In this study, QAC antimicrobial coating inhibited bacterial growth at 0.5% dry weight coating and fungal growth at 3% dry weight coating in denim fabric. These concentrations resulted in 99.4%, 99.5%, 100 % and 100% reductions of bacterial colonies in media plates inoculated with QAC at 0.5%, 2%, 3% and 5%, respectively. Based on the results of our cytotoxicity studies of human skin cells, the maximum concentration of QAC that can be coated on denim fabric was 4% in which more than 90% cell viability was observed. The  $IC_{50}$  value was reached at 13% QAC.

**Keywords:** Antibacterial; Antifungal; Denim; Finish; Quaternary Ammonium Compound

## Introduction

The importance of antimicrobial finishes has become extremely important due to the increase in the usage of reusable clothing as well as the cost of cleaning. These finishes are used to inhibiting odors and contaminants in number of personal care products. These biocidal textiles can play an important role in preventing infectious diseases [1]. Antimicrobial finishes are used to inhibit odors and contaminants in clothing and a number of personal care products. These biocidal textiles can play an important role in preventing infectious diseases [1]. Different microorganisms grow on both natural textile materials like cotton and synthetic material like polyester and polyamide. The properties of textiles like moisture, oxygen and nutrient transport make the fibers a good medium for growth of microorganisms that cause odor and disease [1]. The microbes that can grow on textiles include fungi, bacteria and algae. The bacteria may include both gram positive and gram-negative organisms (Table 1) [2]. There are many advantages of antimicrobial-coated fabrics compared to non-coated fabrics. The antimicrobial finishes can control the infestation of the fabric by microbes, reduce the growth of these microbes, and protect the textiles from staining and discoloration. Antimicrobial finishes also have important medical applications [3], as textiles used in hospitals are can potentially transmit microbial disease. These finishes are important in reducing the spread of disease in hospitals by exposure to contaminated materials [4].

Bacteria	Fungi
<i>Staphylococcus aureus</i>	<i>Aspergillus Niger, Flavus &amp; terreus</i>
<i>Streptococcus faecalis</i>	<i>Chaeromium globsum</i>
<i>Escherichia coli</i>	<i>Penicillium funiculosum</i>
<i>Salmonella typhosa &amp; choleraesuis</i>	<i>Trichophyton interdigitale</i>
<i>Psuedomonas aeruginose</i>	
<i>Mycobacterium smegmatis &amp; tuberculosis</i>	
<i>Stretococcus mutans</i>	
<i>Klebsiella pneumoniae</i>	
<i>Staphylococcus epidermis</i>	

**Table 1:** Examples of micro-organisms known to survive on fabric [2].

Nosocomial infections a great burden to society as they were estimated to cost the US \$4.5 billion and causes 88,000 deaths in 1995 [5,6]. Antimicrobial textiles can be used in both healthcare applications such as wound dressing and in military applications such as biological protective suits [7]. Antimicrobial finishes can also be used in mass market products such as bathing towels, face cloths, pillow covers and undergarments [7]. Different fabrics coated with antimicrobial finishes are used in a variety of fields

and aid in the reduction of disease. Therefore, durability against repeated laundering associated with normal use should be taken into consideration when textiles are treated with antimicrobial finishes [8]. Antimicrobial finished textiles can be categorized into temporary and durable types based on the stability of the antimicrobial properties. In case of temporary antimicrobial finishes, the antimicrobial properties of the textile are lost through laundering. The durability of antimicrobial finish depends on both the kind of antimicrobial finish and type of process used to coat the fabric. The durability of antibacterial agents can be increased via wet finishing processing. Because the antimicrobial fabrics are in close contact with the human body, care should be taken while selecting the antimicrobial agents used for antimicrobial finishes [9]. Some of the characteristics that should be taken into consideration when selecting the antimicrobial finish include, that ability of the compound to inhibit the growth of microbes, the toxicity of the compound to humans, the retention the integrity of the fabric (i.e., color and strength) after the integration of the compound, and the economic viability of the finished fabric for production and sale [9].

Several American Association of Textile Chemists and Colorists (AATCC) approved methods exists to determine the efficacy of the antimicrobial finishes on textiles. In this study, we have employed a number of these techniques to evaluate the utility of the Quaternary Ammonium Compound (QAC) as a suitable textile coating for medical applications. The results indicate that a QAC coating increased the durability of the fabric, maintained antimicrobial properties after application and displayed a cell toxicity tolerance at 4% QAC.

## Materials and Methods

### Coating of QAC on Denim Fabric using Pad-dry-cure Method

Denim samples (100 % cotton fabric saturated with Bourbon dye [indigo/sulfur top]) were obtained from Plains Cotton Cooperative Association (PCCA), Lubbock, Texas. The denim fabric was used to test the efficacy of QAC on microbes and its potential toxicity to human skin in an *in vitro* skin cell model. The denim sample had development style number 2504 (Table 2). The finish was rigid and dark indigo in color designed for maximum retention of a deep rich shade. The samples went through different abrasive washes, including the addition of pumice stones or perlite and/or enzymes, to enhance the appearance. Considering the characteristics of the fabric, the sulfurs contained within the fabric and dye could be directly impacted if alkaline detergent was used and the fabric would desize at temperatures above 130°F. Additionally, hot alkaline peroxide treatment could be used carefully to remove excessive color.

Property	Production	Individual Roll	Standard Test Method
	Average	Minimum	
Construction	68x46	+ or - 2	Standard
Minimum Cuttable Width	N/A	67	Standard
Overall Width including Selvage	N/A	67	Standard
Weight Original (OSY)	11.75	11.2	Standard
3HL Washed Weight (OSY)	11.4	10.9	Standard
Warp Shrinkage Target (%)	-1 to -3	0 to -4	AATCC 135
Filling Shrinkage Target (%)	-1 to -3	0 to -4	AATCC 135
Skew Movement (%)	N/A	-0.03	LS and Co 2
Stiffness Target (lbs.)	7	3 to 11	ASTM D 4032-94
Elongation Target (lbs.)	12	10 to 14	ASTM D 3107-80
Tensile W x F - (lbs.)	184 X 91	140 x 70	ASTM D5034 3H.L.
Tear W x F - (lbs.)	12.5 X 6.75	8 X 5	ASTM D1424 3H.L.
Filling Stretch (%)	N/A	N/A	ASTM D 3107-80
Color Fastness to Laundering	N/A	2	AATCC 61
Rigid Crocking (dry)	N/A	2.5	AATCC 8
Rigid Crocking (wet)	N/A	1.5	AATCC 8
Light fastness - Carbon Arc 20 Hours	N/A	3	AATCC 16

**Table 2:** Properties of denim and its conditioning.

### Conditioning of the Sample

Exposing textiles to high or low humidity affects the moisture pick-up equilibrium of the fabric. This problem can be overcome by conditioning the sample. For conditioning, the room temperature was set at 21°C (70°F) and the relative humidity was set at 65%. The samples were exposed to preconditioning and conditioning atmosphere in which air had free access to the surface area of the fabric by spreading out the material on the shelves of a conditioning rack overnight. Afterward, the samples were taken into a testing room located in a different area than the conditioning room. The conditioned denim fabric was used for experiments 4 min or less after removal from the standard atmosphere.

### Coating of the Sample with QAC

The conditioned sample was cut as per the requirements of the padding mangle. The pickup rate of the vehicle on the denim fabric from the pad bath was determined to be 60% based on the difference between the wet weight ( $m_w$ ) and the dry weight ( $m_d$ ) of fabric divided by dry weight ( $m_d$ ) of fabric to be coated. Pickup rates usually range from 30-100% of liquid on weight of fabric. If the required concentration of QAC antimicrobial on denim fabric was 2% ( $C_d$ ) on the dry weight of fabric, and the pickup rate was 60%, then the QAC Antimicrobial concentration loaded was 3.3% ( $C_r$ , see equation 1).

$$C_d \div \frac{(m_d - m_w)}{m_d} = C_r \quad (1)$$

Different concentrations of the QAC were made in distilled water based on the pickup rate of the denim fabric and the required fabric concentration of QAC. QAC coated concentrations used in this study were 0.5%, 2.0%, 3.0% and 5.0%. These concentrations were achieved by loading the water bath at room temperature with 0.83%, 3.3%, 5%, and 8.3% of QAC, respectively and passing the denim through the bath. This fabric was then passed between two rollers to squeeze out any excess liquid and dried in the drying/ curing equipment at 160°F. This coated denim fabric was used for studying both the physical attributes (i.e., color change, weight change, structural change) as well as the *in vitro* efficacy of QAC against microbes and toxicity in human skin cells.

### Physical Attributes of the Denim Fabric Coated with Antimicrobial Finishes

The change in the physical attributes of color change and percentage of weight loss were determined using a Martindale abrasion tester. The weight loss and color change were studied using American Society for Testing and Materials (ASTM) Test Method D4966. The structural changes were studied using Scanning Electron Microscopy (SEM). Resistance to abrasion of textile fabrics is a very complicated measurement, but it may not include

all the factors related to wear performance or durability of the fabric. Abrasion resistance was stated in terms of the number of cycles and revolutions per minute.

### Martindale Abrasion Test Method

The Martindale test method determines the abrasion resistance of textile fabrics, but difficulties may arise with fabrics with a pile depth of more than 2 mm. Abrasion resistance is measured by subjecting test samples and controls to a rubbing motion in the form of a straight line which gradually becomes an ellipse. Later, it forms another straight line in the opposite direction and traces the same figure again under known conditions of pressure and aberration. Mechanical properties, the dimensions, yarn structure, fabric construction, quantity and type of treatment added to the fibers, abradant nature and action variability of the abradant over the specimen area, specimen tension, and the pressure between the specimen and the abradant are some of the factors that influence the resistance to abrasion in a fabric.

### Conditioning

Appropriate moisture equilibrium is required for testing a denim sample in the standard atmosphere. Equilibrium was attained when there was no substantial increase (i.e., not more than 0.1%) in the mass of the specimen in successive weighing at 2 hrs. intervals. The denim fabric was conditioned in the standard atmosphere for testing textiles, which was 70°F (21°C) and 65 % relative humidity overnight before testing.

### Procedure

All of the tests were conducted in the standard atmosphere. On each testing table, a 140 mm piece of felt was placed followed by a piece of the standard denim fabric of the same size. The mounting weight (supplied with the abrasion machine) was then placed on the table to flatten the fabric/felt pieces. The fabric/felt was securely fastened to the table with the mounting weight in place. The denim sample was weighed to the nearest milligram. As the fabric had a mass/unit area less than 500 g/m<sup>2</sup>, a 38-mm disk of polyurethane foam was placed between the specimen and the metal insert to assemble the holder. The assembled holder was then placed on the machine above the table with the fabric/felt pieces and weight was added to each specimen to exert sufficient pressure. The abrasion machine was configured using the manufacturer's directions after the rotation counter system was set. The test was conducted at a speed of 1.25 x STD (59.4 rpm) which was equal to 59.4 rpm x 1.25 and 100,000 rotations. The post-abrasion specimen was weighed to the nearest milligram, and the percent weight loss post abrasion was calculated.

### Structural Analysis of the Denim Fabric

The antimicrobial treated denim fabric was visualized for

any structural changes in the cotton fabric. SEM was performed on both untreated denim and denim samples treated with 3% and 5% QAC. The SEM was performed on the outside (the dark side) of the fabric. The sample was completely dry when mounted to avoid any traces of water or solvent vaporizing inside the vacuum chamber.

### Assessment of Microbial Resistance

In determining the efficacy of the antimicrobial finishes, the AATCC-147 qualitative test was conducted employing gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). The AATCC-100 quantitative test was performed using gram-negative bacteria *E. coli*. In addition, the efficacy test against fungi *Aspergillus niger* (*A. niger*) was performed using the AATCC-30 protocol.

### Qualitative test (AATCC-147) /Parallel Steak Method

The parallel streak qualitative was used to test the antimicrobial static activity of the QAC coating.

### Procedure

Sterilized nutrient agar was poured into 15 ml in flat-bottomed petri dishes. The agar plates were allowed to solidify before storage. The inoculum was prepared by transferring 1 ml of 24 hrs. broth culture into 9 ml of sterile distilled water in a test tube. The solution was mixed by agitation. A cotton swap was loaded with diluted inoculum and plated on agar plates by making five streaks approximately 60 mm in length and 10 mm apart across the central area of the petri dish. The denim fabric was gently pressed with forceps transversely across the five inoculum streaks to ensure contact with agar surface. The plates were then incubated at 37°C for 18-24 hrs. The same methodology was followed for both the gram-positive bacteria *S. aureus* and gram-negative bacteria *E. coli*. After 18-24 hrs. growth samples were visualized to determine the inhibition zone due to QAC coated denim.

### Quantitative Test (AATCC-100)

Quantitative test was performed against gram-negative bacteria (*E. coli*). The inoculums were prepared following the methods described above for the qualitative test.

### Materials

The treated and untreated (control) denim fabric was cut into 4.8 cm diameter circular swatches using a steel die and placed into 250 ml conical flasks. One swatch was used for each treatment and control.

### Neutralizing Solution

Difco™ D/E neutralizing broth, BD Cat. No. 281910 was used to prepare a neutralizing solution. This solution neutralizes



the antimicrobial chemicals and is used in environmental sampling protocols for the detection and enumeration of microorganisms present on surfaces of sanitary importance. Neutralizing broth was prepared by dissolving in 39 g of the powder in 1L of purified water. The prepared solution was slightly warmed to completely dissolve the powder and then autoclaved at 121°C for 15 minutes.

## Procedure

Using a microliter pipette, one ml of inoculum was added to each swatch in a sterile petri dish. Extra care was taken to make sure that the inoculum was completely absorbed by each swatch. The inoculated swatches were transferred into a 250 ml conical flask and covered to prevent evaporation. Neutralizing solution was immediately added to each flask containing the inoculated untreated control swatch, the inoculated treated test swatches, and the un inoculated treated test swatch. The conical flasks were shaken vigorously for one minute and a serial dilution was performed. A total of 4.5 ml of the media broth was added to 500 ul of each neutralized solution. Afterward, 100 µl of this mixture was transferred onto a petri dish with Trypticase soy agar medium. Petri dishes were labeled and incubated at 37°C for 48 hrs. After 48 hrs. of incubation, the number of colonies were counted on each petri dish. The remaining conical flasks were incubated without neutralizing solution for 24 hrs. followed by the addition of neutralizing solution and serial dilution. 500 µl of the diluted solution was added to the petri dish for incubation (37°C for 48 hours). Number of colonies were counted (24 hours of time point).

## Evaluation

Bacterial counts were reported as the number of bacteria per sample rather than the number of bacteria per ml of neutralizing solution. The percentage reduction of bacteria by denim antimicrobial treatment is calculated by (Equation 2)

$$100 (B - A) / B = R \quad (2)$$

Where

- R= % Reduction
- A= the number of bacteria recovered from the inoculated treated test specimen swatches in the conical flask incubated over 24 hrs. contact time
- B= the number of bacteria recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation that is “0” contact time

## Assessment of Antifungal Activity on Textile Materials:

The Antifungal activity test helps to predict the susceptibility of textile material to mildew and rot and to evaluate the efficacy of fungicides on textile materials.

## Procedure

Denim fabric, both treated and untreated (control), was cut into 2 x 2 cm squares. The fungi species *A. niger* was obtained from American Type Culture Collection (ATCC) no 6275. The fungi were grown on ATCC medium: 336 Potato Dextrose Agar (PDA). The composition included 300 g diced potatoes, 20 g glucose, and 15 g agar in one-liter distilled water. Glucose was added before sterilization and autoclaved at 121°C for 15 minutes.

## Inoculum

A seven to fourteen-day fruiting culture of *A. niger* was grown on PDA and added into a conical flask containing 50 ml of sterile water. One ml of the diluted inoculum was spread over the entire surface area of the petri dish. The test sample was pre-wetted in water and placed on the agar surface prior to even application of 200 µl inoculum using a sterile pipette. The denim containing petri dishes were incubated at 28°C for 7 days. Afterward, the percent surface area of the denim fabric covered with *A. niger* was measured.

## Propagation of Cell line (Hs 733.Sk) for Cytotoxicity Testing

The base medium for the cell line was ATCC-formulated Dulbecco's modified eagle's medium, catalog no. 30-2002. Fetal bovine serum (FBS) was added to the base medium for preparation of complete medium at a 10% concentration in the growth medium. Media was prepared under sterile conditions and at temperature 37.0°C.

## Sub Culturing

The medium was rinsed with 1% PBS (phosphate buffer solution) prior to adding 5 ml of trypsin-EDTA solution. The flask remained at 37°C until the cells were detached. Fresh culture medium was prepared, aspirated, centrifuged. The pellet was re suspended in the growth media and later dispensed into new culture flasks. The cryopreservation for future use was performed in 95% culture medium and 5% DMSO (v/v).

## Method

The cells were cultured in microplates (tissue culture grade, 96 wells, flat bottom) in a final volume of 100 µl/well culture medium in a humidified atmosphere (37°C, 5% CO<sub>2</sub>). The cells were seeded at a concentration of 5 × 10<sup>4</sup> cells/well in 100 µl culture medium into the microplates. The seeded cells were incubated for 24 hrs. at 37°C and 5% CO<sub>2</sub>. After 24 hrs. the normal medium was replaced with medium containing ascending concentrations of QAC. Afterwards, the plates were incubated for 24 hrs. at 37°C and 5% CO<sub>2</sub>. Then, 10 µl of cell proliferation reagent WST-1 was added and the plates were incubated for 4 hrs. at 37°C and 5% CO<sub>2</sub>.

The plates were thoroughly shaken for 1 min. The absorbance of each sample was measured against a background control blank using a microplate (ELISA) reader. The wavelength for measuring the absorbance of the formazan products ranged from 420 - 480 nm. The reference wavelength was 660 nm.

## Results and Discussion

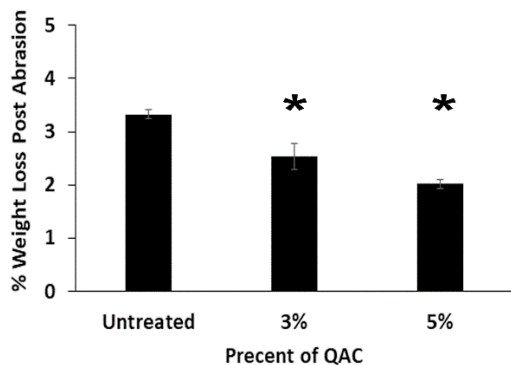
The analysis of end-point abrasion can be determined in different ways. The end-point is typically considered to have been reached when two or more yarns of woven fabrics have broken or when a hole appears on a knitted fabric. The end-point can also be

set to a point in which the shade or appearance of the fabric changes enough to result in a customer complaint [10]. Changes of fabric shade can arise from the loss of a raised finish or changes in effects on fancy yarns. Differential loss of yarn or fiber can cause changes in shade or appearance. The end-point can also be assessed against the AATCC gray scale for color change [11].

To evaluate this change in end-point abrasion, we compared the difference in mass of the fabric swatch before and after abrasion (Table 3, Figure 1). We considered the change in color and weight loss between the treated and untreated fabric.

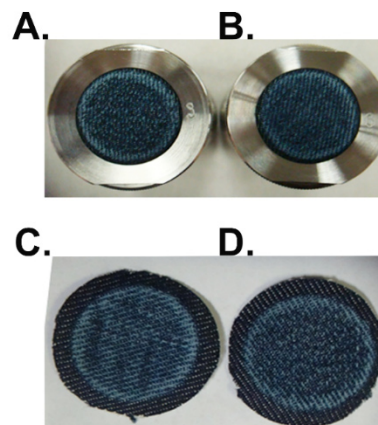
Untreated		3% QAC		5% QAC	
Initial wt(mg)	Final wt (mg)	Initial wt(mg)	Final wt (mg)	Initial wt(mg)	Final wt (mg)
421 ± 4.6	407 ± 4.6	408 ± 3.9	397 ± 4.7	411 ± 2.4	403 ± 2.5

**Table 3:** Abrasion testing of denim.



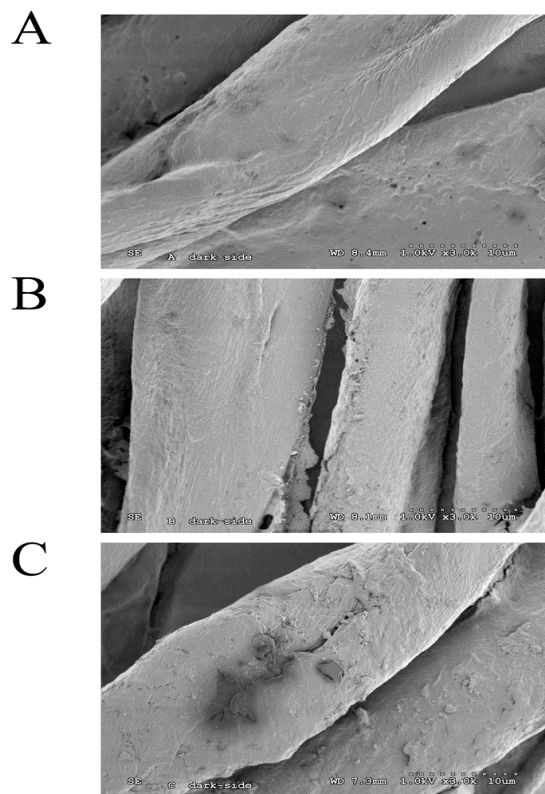
**Figure 1:** Changes in weight of control, 3% QAC treated, and 5% QAC treated samples.

Less weight loss occurred in the denim samples treated with QAC compared to the control or untreated denim samples. The loss of weight in the abrasion test decreased with the increase of percentage of treatment, but the increase was not significant ( $p=0.001$ ; Tukey-Kramer) The weight loss was 2.54% in the sample treated with 3% QAC and 2.03% in sample treated with 5% of QAC. The percent weight loss (2.54% and 2.03%) in the denim samples treated with 3% and 5% QAC respectively, was significantly lower ( $p=0.001$ ;  $p<0.05$ ) than the respective weight losses (3.30% and 3.36%) in the control (untreated denim samples). The reduction in percentage of weight loss due to abrasion in the treated samples may be attributed to smoothing of the fabric surface by the antimicrobial coating.



**Figure 2:** Denim samples showing different color shades after abrasion test (100,000 rotations) in areas exposed and unexposed to abrasion. Untreated (A and C) and QAC 5% treated (B and D).

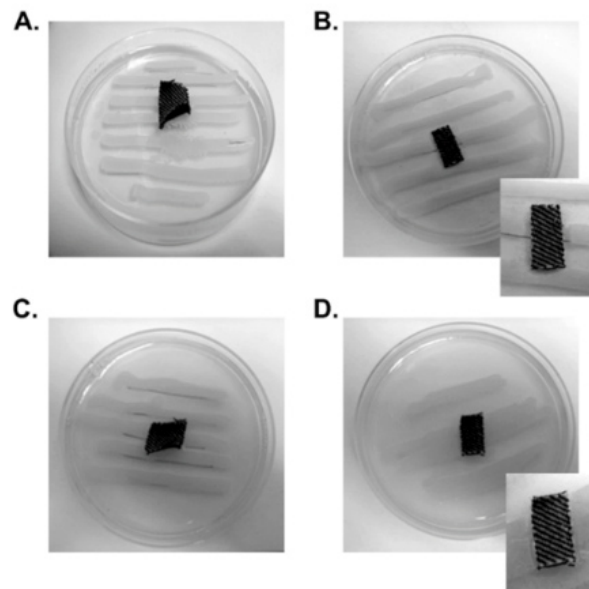
Changes in the shade of the denim sample were noted after the abrasion test. The change in shade was observed in both the untreated and treated samples (Figure 2). These changes could be attributed to the friction between the testing and standard samples and the abrasive surface. SEM results indicated uniform coating of 3% and 5% QAC treatments of the cotton fibers (Figure 3). Unlike zinc oxide nanoparticles (ZnO NPs) treated cotton fibers, there were no crystals or formation of granules in the QAC treated samples [12].



**Figure 3:** A) SEM of the outside (dark side) of the untreated denim fabric. B) SEM of the outside of the denim fabric treated with 3% of QAC. C) SEM of the outside of the denim fabric treated with 5% of QAC.

### Qualitative test (AATCC-147) /Parallel Steak Method

Using the parallel steak method, we tested the bacterial strains *S. aureus* and *E. coli*. There was a clear growth inhibition of bacteria in gram-positive bacteria *S. aureus* at the area of contact between the bacteria and the denim fabric (Figure 4). A clear zone of inhibition was not seen. This may be attributed to the non-leaching or non-diffusion of the QAC into the medium from the denim fabric. If there was diffusion of the QAC, growth inhibition zones would be present away from the source (denim fabric). Considering the absence of this diffusion, the finish is likely high durability. Growth inhibition was comparable in both *E. coli* (Figure 4) and in *S. aureus*.

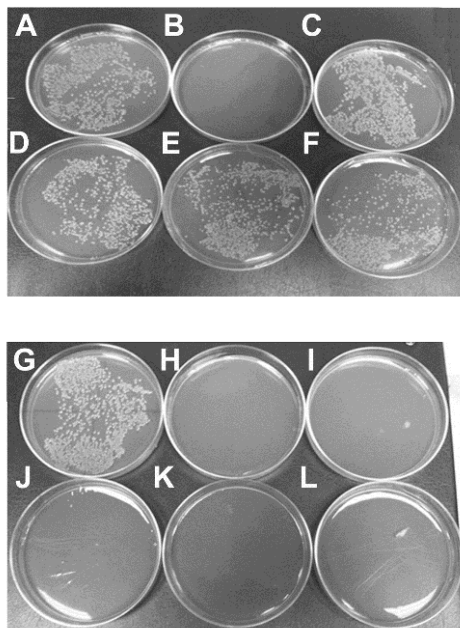


**Figure 4:** Antimicrobial efficacy test (Parallel Streak method) using *S. aureus* against denim samples A) untreated or B) treated with 0.5% QAC. The antimicrobial efficacy test using *E. coli* against denim samples C) untreated or D) treated with 0.5% QAC.

### Quantitative Test (AATCC-100)

The AATCC-100 quantitative test was performed using the gram-negative bacteria *E. coli*. Colonies were counted 24 hrs. after plate inoculations. The inoculum was exposed to the denim samples for 0 or 24 hrs. (Figure 5) shows the 0 hr. (A-F) and 24 hrs. (G-L) plates after brief (0 hr.) and 24 hrs. exposure to denim. Table 4 shows the quantitation of the plates displayed in Figure 5 and the percent reduction in the number of colonies in various conditions. *E. coli* proliferated upon exposure to untreated denim (-32.7% reduction), and no growth occurred in the uninoculated plate. There was a 99.4%, 99.5%, 100% and 100% reduction in colonies in the plates treated with 0.5%, 2%, 3%, and 5% of QAC, respectively (Table 4). There was a significant reduction ( $P < 0.0001$ ; Tukey-Kramer) in *E. coli* colonies at 0.5% QAC treatment after 24 hours. This implies that the minimum concentration required for the mitigation of bacterial growth is 0.5% of QAC (Table 4).





**Figure 5:** *E. coli* inoculum plated after brief 0 hr. contact time with denim fabric treated in different conditions. Top from left: A) untreated inoculated, B) treated not inoculated, C) treated with 0.5% QAC and inoculated. Bottom from left: Inoculated and treated with D) 2%, E) 3%, F) 5% QAC respectively. *E. coli* inoculum plated after 24 hrs. contact time with denim fabric treated with different percentages of QAC Top from left: G) untreated inoculated, H) treated not inoculated, I) treated with 0.5% QAC and inoculated. Bottom from left: Inoculated and treated with J) 2%, K) 3%, L) 5% QAC respectively.

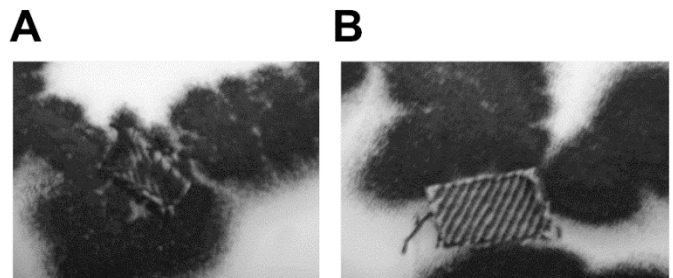
QAC treated Denim fabric	E.coli colonie count "0" hrs contact time	E.coli colonie count "24" hrs contact time	% Reduction(R) R=100(B-A)/B
	(B)	(A)	
untreated inoculated	566	751	-32.70%
Treated not inoculated	0	0	-
Treated with 0.5%	546	3	99.40%
Treated with 2%	387	2	99.50%
Treated with 3%	394	0	100%
Treated with 5%	396	0	100%

**Table 4:** Growth inhibitions of denim treated QAC.

### Assessment of Antifungal Activity

The growth of fungi was observed after seven days of incubation at 28°C, but there was no growth of fungi on the denim fabric treated with QAC. There was, however growth of fungi on the untreated (control) denim fabric (Figure 6). It was also observed that QAC is less effective against fungi than bacteria. At 3% QAC denim fabric treatment, complete growth inhibition of fungi was observed. The effect was very prominent at the 5% treatment. Like bacteria, there was an indistinct zone of fungal growth inhibition

due to the non-leaching characteristics of QAC.

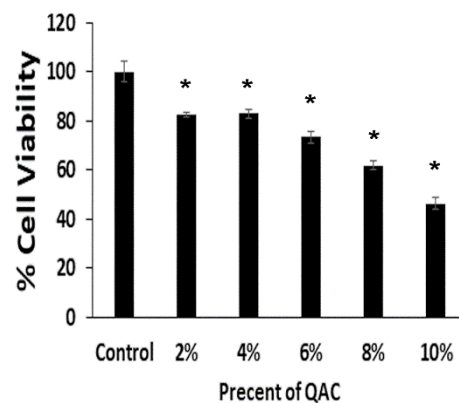


**Figure 6:** *Aspergillus niger* growth on denim fabric A) untreated B) Treated with 3% of QAC.

### Assessment of Cytotoxicity in Human Cells

In the 96 well plates, after cytotoxicity testing of the Hs773Sk cell line after 24 hrs. of treatment, there were approximately 90% viable cells at 2% and 4% of QAC treatment (Figure 7). At 6% of antimicrobial, the viability of cells was reduced to 80%. The IC<sub>50</sub> value is predicted to be at 13 % of QAC treatment when calculated using probit analysis in SPSS software.

We selected a 90% cell viability threshold to determine cytotoxicity. Based on this the maximum concentration of QAC that can be used to coat the denim fabric is 4%. (Figure 7) shows the percentage of viable cells at different gradients of QAC (Figure 7). It is possible to use higher concentration of the QAC in the area where there is no direct contact with human skin. This problem can be solved by using one more layer of non-treated clothing inside of the protective suits developed from this treatment. These protective suits would be more suited for mitigating the effect of microbes from external source.



**Figure 7:** Percentage human normal skin fibroblast viability with increasing concentrations of QAC.



## Summary

Different concentrations of QAC were successfully coated onto the denim fabric which was 100% cotton. The coated fabric was used to understand the different biotic and abiotic changes that can occur in treated samples in comparison to untreated or control samples. Different abiotic tests that were conducted to understand the physical attributes were weight loss test, aberration, and an analysis of visual structural alterations using SEM on denim samples treated with QAC antimicrobial. The biotic properties studied were assessment of microbial resistance and cytotoxicity studies using Hs773Sk cell line.

The coating of the denim with QAC appears to be a plausible approach for sterile applications in various applications. The apparent absence of leaching of the QAC from the fabric provide great indicators that QAC coating maintains durability on the denim fabric. These properties designate QAC coating a viable application for fabric coating within the health field to prevent the spread or development of microbial infections.

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