THESIS

A COMPARATIVE EVALUATION OF ANTIMICROBIAL PROPERTIES AND DURABILITY TO LAUNDERING OF SELECTED ANTIMICROBIAL AGENTS ON A HOSPITAL TEXTILE

Submitted by

Anupama Sargur Ranganath

Department of Design and Merchandising

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Master's Committee:

Advisor: Ajoy K. Sarkar

Dawn M. Mallette
Juyeon Park

ABSTRACT

A COMPARATIVE EVALUATION OF ANTIMICROBIAL PROPERTIES AND DURABILITY TO LAUNDERING OF SELECTED ANTIMICROBIAL AGENTS ON A HOSPITAL TEXTILE

The Centers for Disease Control and Prevention, USA estimates that approximately 1.7 million Healthcare Associated Infections (HAIs) and 99,000 associated deaths occur each year on account of infection-causing bacteria. Hence, the control of infections has been identified as the most important target by the United States Department of Health and Human Services. HAIs can be minimized by inhibiting the various routes of transmission of bacteria. Textile substrates have been implicated as one of the vectors of transmission of disease. The spread of infection causing bacteria via textile materials is inhibited by the use of antimicrobial treated textiles. Based on an exhaustive literature review on antimicrobial textiles, it was found that a majority of the research conducted to-date has focused on synthesizing and evaluating uniquely distinct antimicrobial agents on different textile substrates with the main aim of proving their effectiveness against microbes. Very few studies have concentrated on comparing the durability to laundering and antimicrobial efficacy of different agents on a specific substrate against target challenge microorganisms.

The present research compared the efficacy and durability to laundering of five antimicrobial agents of distinctive antimicrobial chemistries and modes of action on a polyester-cotton substrate. The antimicrobial agents were based on silver, triclosan, QAC, PHMB and chitosan. The challenge microorganisms were *Staphylococcus aureus*, a gram positive bacterium and *Escherichia coli*, a gram negative bacterium. Specimen samples of the polyester-cotton substrate treated with the antimicrobial agents were subjected to a maximum of fifty wash cycles and subsequently evaluated using standard qualitative and quantitative test methods. Scanning Electron Microscopy analysis of the treated and laundered substrates was done to study the difference in topography of the substrates. Statistical analysis for comparing the antimicrobial properties and durability to laundering of the antimicrobial treated fabrics was done using Statistical Analysis System.

Qualitative results showed that the triclosan-based antimicrobial agent had superior durability to laundering than the other controlled release antimicrobial agents in this study. SEM analysis of the treated and laundered substrates at ten and fifty wash cycles revealed no visible differences in the topography of the specimen samples. In agreement with qualitative data, quantitative results indicated that triclosan was most effective against both *E.coli* and *S.aureus* after fifty wash cycles. Silver, QAC, PHMB and chitosan had higher efficacy against *S.aureus* than against *E.coli*. The antimicrobial action of silver, QAC, PHMB and chitosan decreased with increase in number of laundry cycles and the decrease was more pronounced against *E.coli*.

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Chapter 1

INTRODUCTION

Microorganisms have occupied every habitat on earth: from geothermal vents to the coldest Arctic ice. They play both beneficial and harmful roles in our lives. Some of the beneficial roles include production of oxygen via photosynthesis, nitrogen fixation, circulation of carbon by decomposition of dead organic matter, formation of crude oil, and helping animals such as cows digest their food. They are used by humans in making bread, beer, cheese, and antibiotics. Some of the harmful effects are caused by the virulence of pathogenic microorganisms, i.e., infection causing bacteria such as *Staphylococcus aureus* (*S.aureus*), *Escherichia coli* (*E.coli*), and *Enterococcus faecalis* (*E.faecalis*). An outbreak of meningitis, in Fort Collins, CO, USA during June 2010 was a bacterial infection that spread through contact (http://www.thedenverchannel.com/news/23956981/detail.html). The Centers for Disease Control and Prevention (CDC, USA) estimates that approximately 1.7 million Healthcare Associated Infections (HAIs) and 99,000 associated deaths occur each year on account of infection-causing bacteria. About 85% of all invasive methicillin-resistant *Staphylococcus aureus* (MRSA) infections were associated with health care (Klevens et al., 2007). In 2005, there were about 94,360 people who developed a serious MRSA infection in the United States of whom 18,650 people died. Hence, the control of infections has been identified as the most important target by the United States Department of Health and Human Services (HHS). The department has developed an action plan to prevent healthcare associated infections by identifying targets and metrics for five categories of HAIs listed below

(http://www.cdc.gov/ncidod/dhqp/stateHAIplan.html):

- Central line associated blood stream infections
- *Clostridium difficle* infections
- Catheter associated urinary tract infections
- Methicillin resistant *Staphylococcus aureus* infections
- Surgical site infections

Health care associated infections can be controlled by inhibiting the various routes of transmission that causes an infection to spread from an infected person to healthy person. The various routes through which an infection can spread are direct contact with infected individuals; infected water and food; contact with inanimate objects such as textiles used in scrubs, doctor's coats, surgical gowns, bed-sheets, pillow covers, and curtains.

The control of the spread of infections via infected individuals, water and food can be achieved by developing hygienic practices. The spread of infections through textile materials can be controlled by the use of *antimicrobial textiles* that kill pathogens on contact or hinder their ability to reproduce prior to being transferred on to another material or person. It is also pertinent to mention that other than the requirements of the healthcare facilities; the increase in consumer's demand for comfort, hygiene and well-being has created a large and rapidly increasing market for antimicrobial textiles (Gao & Cranston, 2008). As an example, the market for disinfectants and antimicrobial chemicals in the US is expected to rise by 5% annually (Freedonia group, 2009).

Antimicrobial textiles are made by treating textile substrates with antimicrobial agents. Antimicrobial agents are bound to textiles by different methods depending on the chemistry between the antimicrobial agent and the textile (Gao & Cranston, 2008). The most widely used antimicrobial agents for textile applications are based on metal salts (for e.g., silver), quaternary ammonium compounds (QAC), halogenated phenols (for e.g., triclosan), polybiguanide (for e.g., PHMB), chitosan, and N-halamines. These antimicrobial agents have been studied independently

and have been proven to possess effective antimicrobial ability by previous researchers (Simoncic & Tomsic, 2010). Other than the antimicrobial ability, there are certain basic requirements to be satisfied by an antimicrobial agent for its successful application on textiles rendering them to be used commercially. The basic requirements of a good antimicrobial agent for textile substrates are summarized below (Gao & Cranston, 2008; Kramer et al., 2006; White & Montecello, 2002):

- Should possess affinity for specific fabric and fiber types.
- Be easy to apply on textile substrates.
- Be able to inactivate undesirable microbes while simultaneously not affect desired microbes.
- Inert to chemicals to which the textile might be exposed during processing.
- Durable to repeated laundering, dry cleaning, ironing and prolonged storage including resistance to detergents used to care for the textiles.
- Stable during usage without degrading into hazardous secondary products.
- Not adversely affect the user or the environment.

Based on an exhaustive literature review on antimicrobial textiles, it was found that most of the research conducted to-date has focused on synthesizing and evaluating uniquely distinct antimicrobial agents on different textile substrates with the main aim of proving their effectiveness against various microbes. Previous research has thus resulted in the availability of a variety of antimicrobial textiles (Gao & Cranston, 2008; Simoncic & Tomsic, 2010). However, very few experimental studies have been reported that compare the performance of different antimicrobial agents with regard to their durability on textile substrates. The durability characteristics of antimicrobial treated textiles is of considerable importance since textile materials used in healthcare surroundings are frequently refurbished under harsh industrial or hospital laundry conditions. A comparison between the antimicrobial agents will potentially yield valuable information and help in cataloging the agents according to their efficacies and durability.

This classification, in turn, will be useful in selecting antimicrobial agents more efficiently and according to the requirement of different sectors of the healthcare community as well as for other end-users among the general population. Effective usage will subsequently lead to mitigating the abuse of antimicrobial compounds in wide use.

The purpose of this research was to investigate the most effective and durable antimicrobial treatment for a representative textile substrate, a polyester/cotton blend, that is widely used in healthcare environments. The research focused on the comparison of five selected antimicrobial agents chosen on the basis of their wide use, novel chemistries and diverse modes of action. The selected agents were:

- Silver
- Triclosan
- Quaternary Ammonium Compound (QAC)
- Polyhexamethylene Biguanide (PHMB)
- Chitosan

The effectiveness and durability of the antimicrobial agents were examined via their activity against two microorganisms most responsible for infections viz. *E.coli* and *S.aureus*. The specific objectives of the research were:

- Study and compare the efficacy of five antimicrobial agents with distinctive chemistries and diverse mode of action on a polyester/cotton blend substrate.
- Compare the durability properties of the five antimicrobial agents on the treated test substrate after treatment and post-laundering at 10 and 50 wash cycles.

Null hypotheses:

1. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *S.aureus* at "0" wash cycles (before laundering).

2. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *E.coli* at "0" wash cycles (before laundering).
3. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *S.aureus* after 10 wash cycles.
4. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *E.coli* after 10 wash cycles.
5. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *S.aureus* after 50 wash cycles.
6. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *E.coli* after 50 wash cycles.

Chapter 2

LITERATURE REVIEW

Textiles are among the most widely used materials in everyday use. The end-use of a textile material dictates its desirable properties. Properties of a textile material are determined by the chemical nature of the fiber in the material, for e.g., cotton is composed of cellulose which makes it more absorbent; polyester is composed of polyethylene terephthalate which makes it a strong fiber. Consumer's demand for better performance has led to the incorporation of additional properties to existing fiber types. Chemical treatment of fabrics is one way of adding special properties without hampering the inherent nature of the fabrics. For example, a cotton fabric is comfortable next-to-skin but is flammable. Cotton can be made flame retardant by an appropriate chemical finishing treatment. Other finishes have been developed for application on a wide range of textile materials including treatments for permanent finish, anti-static; anti-UV and anti-microbial among others. Textiles can therefore be designed for special functional uses for applications in defense, firefighters and healthcare environments.

2.1. Healthcare Textiles

Textiles used in health care environments are required to possess antimicrobial property to minimize spread of infection. Anti-microbial property can be imparted via chemical finishing with an antimicrobial agent. The sections that follow describe the different antimicrobial agents based on their mode of action (Shindler & Hauser, 2004).

2.2. Antimicrobials with controlled release or ‘leaching’ mechanism

The antimicrobial agents that belong to this category do not form strong bonds with the textile substrate. The chemical species responsible for biocidal activity are released slowly from the treated fabric surface, thus killing all the microbes surrounding the agent. An advantage of leaching antimicrobials effect are their superior antimicrobial activity than compounds based on other modes of action on the same fabric under similar environmental conditions (Kut, Orhan, Gunesoglu & Ozakin, 2005). The flip side is that the antimicrobial agent in the textile substrate is depleted eventually and loses its effectiveness (White & Montecello, 2002). Metal salts (e.g., silver) and halogenated phenols (e.g., triclosans) are examples of antimicrobial agents that utilize the leaching mechanism (Shindler & Hauser, 2004).

2.2.1. Metal salts (silver, copper, and zinc)

Silver based antimicrobial agents are broad spectrum antibiotics and are one of the oldest and most widely used biocides. In the presence of moisture silver releases ions which bind the bacterial cell’s surface with proteins (Figure 1). On binding, the following action occurs (Feng, et.al., 2000):

- Denaturing effect of the silver causes DNA to get condensed and lose its replication abilities.
- Induces inactivation of bacterial proteins by reacting with thiol group (Feng, et.al., 2000).

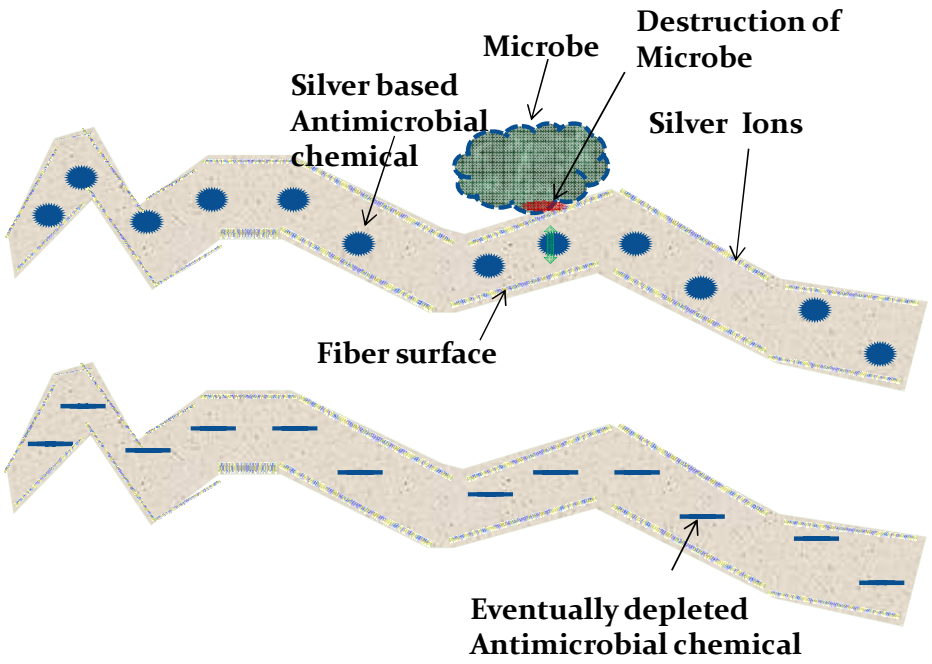

Figure 1: Illustrative antimicrobial mechanism of a silver-based antimicrobial agent.

Silver is effective at low concentrations and promotes wound healing without appreciable toxic risk. However, there is a small risk of developing allergies to silver compounds (Lansdown, 2002; Lansdown, 2004). Other metal based antimicrobial agents found to exhibit good antimicrobial properties are based on copper and zinc compounds, in the form of their sulfides and sulfates (Nakashima, Sakagami, Ito & Matsuo, 2001). Recent studies on metal salts have focused on preparation of nano sized metal particles, which has led to the development of new generation of biocides (Simoncic & Tomsic, 2010).

2.2.2. Halogenated phenols (Triclosan)

Triclosan, a chlorinated phenolic compound is a derivative of a diphenyl ether compound (Figure 2). Triclosan is a component in many consumer health care products such as soaps, detergents, hand wash, textiles and household objects (Simoncic & Tomsic, 2010).

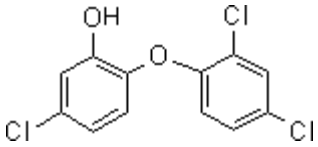

Figure 2: Structure of Triclosan (Gao & Cranston, 2008)

Triclosan inhibits the growth of microbes by using an electrochemical mode of action to penetrate and disrupt the cell wall of microbes. When incorporated within a polymer, it migrates to the surface and protects the material (Gao & Cranston, 2008; Mansfield, 2002). When embedded in β-cyclodextrin triclosan forms a complex and can exhibit antimicrobial action with minimum quantities (Lu et al., 2001). Some researchers claim that triclosan inhibits a specific function i.e., lipids synthesis in a bacteria (Mcmurry, Oethinger & Levy, 1998). Others claim that lower levels of triclosan resistance by strains of bacteria shows that triclosan inhibits bacterial cell function in multiple ways. A decrease in the antimicrobial efficiency of triclosan treated material when the material is subjected to repeated home wash cycles has been reported by Orhan, Kut & Gunesoglu (2007).

2.3.Bound or non-leaching type antimicrobials

The antimicrobial agents that belong to this category are chemically bound to the textile substrate. Hence, the antimicrobial can act only on the microbe that comes in contact with the treated textile's surface. By virtue of its binding nature, these antimicrobials do not get depleted and therefore potentially may have higher durability than leaching mechanism antimicrobials (Malek & Speier, 1982; White & Montecello, 2002). However, compounds on a treated fabric might get abraded or deactivated with long term usage and lose their durability (Shindler & Hauser, 2004). The antimicrobial agents listed under this category are Quaternary Ammonium Compounds (QACs), Polyhexamethylene Biguanide (PHMB), chitosan and N-halamines.

2.3.1. Quaternary Ammonium Compounds (QACs)

QAC's typically possess a silane base at one end of the molecule and a long molecular chain of carbon atoms at the other end (Figure 3).

In a fabric treated with a QAC based antimicrobial agent, the silane base of the compound reacts with the fabric and forms a covalent bond. The other end is projected out and is positively charged. When a microbe approaches the fabric the free end of the agent's molecule reacts with the cell wall and causes a leakage of the negatively charged species in the microbe cell. It eventually causes the cell's death (Malek & Speier, 1982; Mulder, Cavorsi & Lee, 2007).

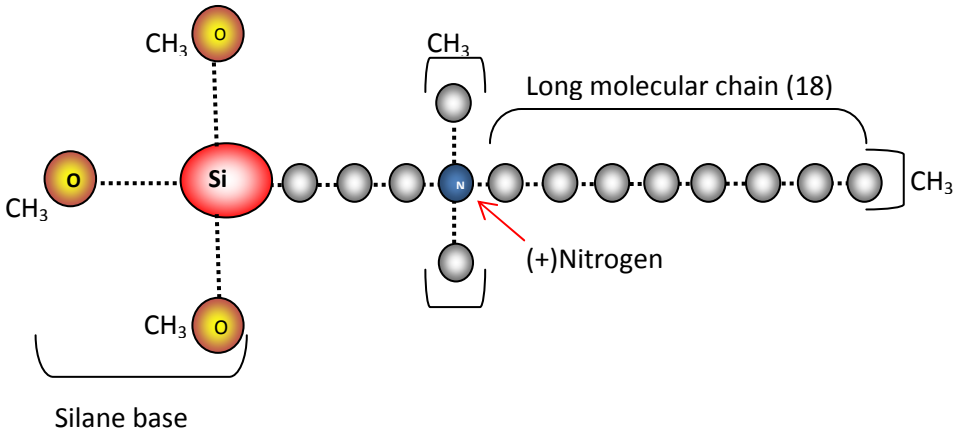

Figure 3: Structure of a Quaternary Ammonium Compound

Dyes can be used as a link between quaternary ammonium salts and synthetic fabrics. Hence a dyed fabric can achieve higher add on levels of QACs and antimicrobial efficacy as compared with undyed fabrics (Kim & Sun, 2000).

2.3.2. Polyhexamethylene biguanide (PHMB)

PHMB is a hetero disperse mixture of polyhexamethylene biguanide (Figure 4). The halide form of PHMB i.e., polyhexamethylene biguanide hydrochloride is applied on cellulosic materials (Payne & Yates, 2007).

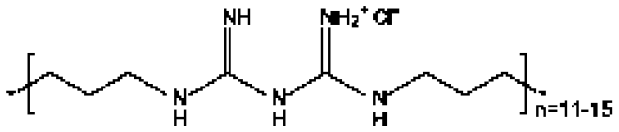

Figure 4: Structure of PHMB (Gao & Cranston, 2008)

PHMB is found to form hydrogen bonds with cellulosic fibers. With the increase in the concentration of PHMB there is a dominant increase in hydrogen bond formation between PHMB and fibers (Blackburn, Harvey, Kettle, Payne & Russell, 2006). When the fabric treated with PHMB comes in contact with a bacterium, the biocide interacts with the surface of the bacteria and is transferred to the cytoplasm and cytoplasmic phospholipids in the bacterial membrane. This biocide is positively charged, and therefore it mainly reacts with negatively charged species and includes aggregation, leading to increased fluidity and permeability. This results in the leakage of inner material from the outer membrane and eventually causes death of an organism (Mulder, et al., 2007).

2.3.3. Chitosan

Chitin, a poly (β-(1-4)-N-acetyl-D-glucosamine) is a natural polysaccharide (Figure 5). Chitin is synthesized by many living organisms. It is the most abundantly found polymer second only to cellulose. When chitin is acetylated to at least about 50%, then it is called chitosan (Rinaudo, 2006).

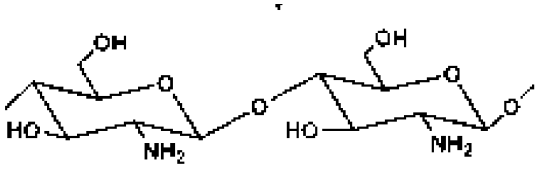

Figure 5: Structure of Chitosan (Gao & Cranston, 2008)

Chitin is a film forming polymer with antibacterial and fungi-static property. It triggers the defensive mechanism in host inducing certain enzymes like phytoalexins, chitinases, pectinases, glucanases, and lignin in plants (Rinaudo, 2006). One of the potential problems with an effective chitosan based antimicrobial agent is that chitosan is insoluble in water and possesses high molecular weight. The high molecular weight increases the viscosity of the medium and causes detrimental effect on the hand and feel of the fabric (El-tahlawy, El-bendary, Elhendawy & Hudson, 2005). Mechanism studies suggest that the positively charged chitosan interacts with negatively charged residues at the cell wall of fungi or bacteria. The interaction changes cell permeability and causes the leakage of intracellular substances (Lim & Hudson, 2004; Young, Kohle & Kauss, 1982). Other studies suggest that the formation of the polymeric substance around the bacterial cell prevents the nutrients from entering the cell (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades & Roller, 2001).

2.3.4. N-halamines

N-halamines are heterocyclic compounds containing one or two covalent bonds formed between nitrogen and halogen. Typical chemical formulae are as shown in Figure 6: Monomethylol-5,5-dimethylhydantoin (MDMH) or Dimethylol-5,5-dimethylhydantoin (DMDH) (Lin, Winkelman, Worley, Broughton & Williams, 2001). The halogen, which is usually chloride, is replaced with hydrogen in presence of water or chloroform and acts as biocide (Qian & Gang, 2005).

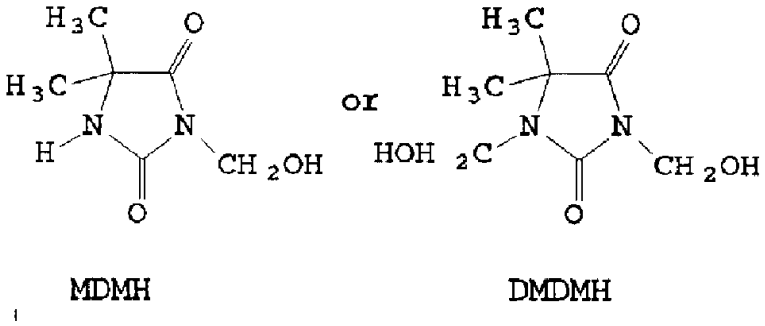

Figure 6: Structure of N-Halamines

When the bound chlorine atom comes in direct contact with the bacterial cell, it results in the transfer of chlorine and subsequently causes cell inactivation (Lin, et al., 2001). It has also been found to have extraordinarily durable biocidal functions in a series of laundering tests (Sun, Xu, Bickett & Williams, 2001). However, N-halamine materials are found to be decomposed upon exposure to ultraviolet irradiation as in direct sunlight (Kocer, et.al, 2010).

2.4.Gaps in current literature

Most studies reported to-date have focused on studying the effects of varying concentrations of a particular antimicrobial agent on a specific textile material. This was done to determine the optimum concentration required to obtain good antimicrobial efficacy. Additionally, the studies were carried out to observe the behavior of antimicrobial agents with variation in pH and temperature. Lastly, many new chemical compounds with antimicrobial properties were developed in the laboratory and tested for antimicrobial efficacy (Zhao & Sun, 2006; Zhao & Sun, 2007). Even though there have been successes in achieving good antimicrobial properties in the laboratory, there are some drawbacks as follows:

- Textiles used for various purposes are laundered regularly to maintain hygiene. Laundering of treated textiles depletes quantity of antimicrobial chemicals on the fabrics

and hence reduces protection. The antimicrobial efficacy reduces with the increase in number of laundry cycles and subsequent increase in the cost of re-application.

- With increase in the usage of antimicrobial agents, it is found that bacteria are becoming resistant to antimicrobials.

Therefore, there is a need for additional research that addresses the drawbacks. The current research seeks to find answers by investigating the different antimicrobial agents with regard to their efficacy and durability to harsh laundry conditions. A comparison between different antimicrobial agents under similar laundering conditions will enable them to be organized in terms of their antimicrobial efficacy with reference to durability to laundering.

Chapter 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Substrate

The fabric used for this research was a blend of 35% cotton/ 65% plain weave polyester with the following characteristics - weight: 154 g/m^2; fabric count: 158; thickness: 0.017 inches. This blended fabric is one of the most widely used textiles in health care environments. The fabric was purchased from Testfabrics, Inc. Pennsylvania, USA [Style #7436].

3.1.2. Antimicrobial agents

3.1.2.1. Silver

The silver based antimicrobial agent was composed of a mixture of silver chloride and titanium dioxide and can be applied to all textile fibers with the exception of peptide based fibers. It has biostatic activity against most gram positive and gram negative bacteria as well as some yeast and micro-fungi.

3.1.2.2. Triclosan

The antimicrobial agent based on triclosan was a halogenated phenol with the chemical constitution 5-chloro-2-(2.4-dichlorophenoxy) phenol. It is the most widely used biocide in health care and household products (Simoncic & Tomsic, 2010).

3.1.2.3. Quaternary Ammonium Compound

The quaternary ammonium based antimicrobial agent was based on 3-trimethoxysilypropyldimethyloctadecyl ammonium chloride (Simoncic & Tomsic, 2010).

3.1.2.4. Polyhexamethylene Biguanide (PHMB)

A high molecular weight aqueous solution of 20% PHMB antimicrobial developed specially for textiles was used in this research. This formulation is suitable for cellulosic fibers and its blends with minimum 35% cellulose content.

3.1.2.5. Chitosan

The formulation used for this research contained 6% chitosan as an antimicrobial agent.

3.1.3. Microorganisms

Staphylococcus aureus (ATCC 6538; PML Microbiologicals®) a gram positive bacteria was selected for this research, based on the five categories of HAIs (http://www.cdc.gov/ncidod/dhqp/stateHAIplan.html). Members of the Staphylococcus genus are facultative anaerobic, non-motile, gram-positive cocci. They are 0.5-1.5µm in diameter, occurring singly, in pairs, in tetrads, and characteristically dividing in more than one plane to form irregular clusters. It is normally associated with skin, wound infections, and food poisoning (Willey, Sherwood & Woolverton, 2010). The second bacterium used was a gram negative bacterium, *Escherichia coli* (ATCC 8739). It is a rod shaped bacterium of average size, 1.1 to 1.5µm wide by 2.6 to 6.0 µm length. It is facultative anaerobic bacterium. Some strains have motility. It is a part of human being's normal flora till it gains

virulence factors. When it becomes virulent, it releases toxins and causes severe food poisoning.

3.2. Methods

3.2.1. Treatment with silver-based antimicrobial

The substrate was first washed to remove dust and other impurities and subsequently treated with a silver based antimicrobial agent using the exhaust procedure. The treatment bath was prepared with Material to Liquor Ratio (MLR) of 1:10 and concentration of 0.6% of antimicrobial agent on weight of fabric (owf). The substrate was introduced in the bath with wetting agent at room temperature. The pH of the bath was slightly acidic (up to ~6). After the substrate equilibrated for 5 minutes, the temperature of the bath was increased to 60^{o} C in 10 minutes and maintained at 60^{o} C for 45 minutes. The substrate in the bath was stirred every 5 minutes over the duration of treatment. It was then removed from the bath at room temperature followed by curing at 120^{o} C for 5 minutes.

3.2.2. Treatment with triclosan

The substrate was washed to remove dust and other impurities. Exhaust procedure was adopted to treat the substrate with triclosan. The concentration of triclosan used was 4% owf with a MLR of 1:50. As per the MLR calculations, required amount of water was measured in a treatment bath and heated to 50^{o} C. Triclosan was added to the bath and pH was maintained between 4 and 6. The substrate was then added to the bath and temperature increased to 120^{o} C. Treatment was continued at this temperature for 60 minutes. Finally fabrics were rinsed and air dried.

3.2.3. Treatment with quaternary ammonium compound (QAC)

The substrate was washed to remove dust and other impurities followed by treatment with a QAC antimicrobial using the exhaust procedure. The treatment was started with the required amount of distilled water calculated as per 1:10 MLR in the treatment bath. One percent of the QAC antimicrobial agent on weight of the substrate was measured and immediately diluted with 1:6 parts of water. The water thus used for dilution purposes was pipetted from the measured quantity from the same glass beaker and was mixed with constant stirring for uniform distribution. The diluted mixture was then added and mixed in the beaker with the required amount of water. Absence of turbidity was an indication that the correct procedure was being followed. The pH of the bath was maintained in the range of 4.5 to 6. The substrate was then introduced in the prepared bath and temperature was increased to 50^{o} C. The treatment was continued for 20 minutes at 50^{o} C. In the last step, the bath was allowed to cool to room temperature, substrate removed from the bath and cured at 120^{o} C for 5 minutes.

3.2.4. Treatment with PHMB

The substrate was washed to remove dust and other impurities and treated with PHMB based antimicrobial agent using the exhaust procedure. The MLR for this treatment was 1:10 and concentration of PHMB was 2% on weight of fabric. The calculated amounts of water and PHMB were combined to prepare the treatment bath. The pH of the bath was adjusted to 6-8 with sodium hydroxide. The substrate was introduced at room temperature and the temperature of the bath increased to 40^{o} C. The treatment was continued for 30 minutes. The substrate was then rinsed and air-dried.

3.2.5. Treatment with chitosan

The substrate was washed to remove dust and other impurities. Treatment of the substrate with a chitosan based antimicrobial agent was done by the pad-cure method. In this method, the chitosan based antimicrobial was diluted with 20 parts of water for 1 part of chitosan. The diluted solution was considered as the stock solution. The optimum concentration for the treatment was 2% on weight of the stock solution. The bath was prepared with the required amount of water as per MLR of 1:20 and required amount of stock solution of chitosan. The pH of the bath was maintained between 4.5and 5.5 for the entire duration of treatment. To increase the affinity and durability of chitosan for the substrate, 0.4% of a binder was also added to the treatment bath. The substrate was then immersed in the bath for 5 minutes at room temperature followed by one nip through the squeezing rollers to remove any excess liquor. The substrate was then cured at 149^{o} C in the curing chamber for 5 minutes.

3.2.6. Evaluation of antimicrobial activity

The first method of evaluation of antimicrobial efficacy was qualitative analysis for the presence of antimicrobial activity of treated fabrics. The second method was quantitative analysis to determine percentage reduction in bacteria on treatment by the different antimicrobial agents. Both test procedures require a growing medium to provide ample food for the bacteria to thrive. There are two types of mediums, nutrient broth and nutrient agar. Broth is a liquid medium and agar is a gel that solidifies at room temperature. For the preparation of the nutrient broth, a mixture of 2.5 grams of Bacto™ Peptone, 1.5 grams of beef extract, and 4 grams of sodium chloride were boiled in 500 ml of distilled water for uniform dispersion. For preparing nutrient agar solution, 1.5 % of Difco™ Nutrient agar was

added to the broth above and boiled for a minute. Nutrient broth and agar were then autoclaved for sterilization purposes prior to use.

3.2.6.1. Qualitative evaluation

The qualitative evaluation was carried out using AATCC Test Method 147: Antibacterial Assessment of Textile Materials: Parallel Streak Method. Rectangular test specimens of size 25 X 50 mm were used for the evaluations. Sterilized nutrient agar at 47 o C was dispensed in the petri dishes and allowed to gel firmly. A loopful of the culture was transferred to the surface of the sterile agar plate by making five streaks approximately 60 mm in length, spaced 10 mm apart covering the central area of the petri dish. The specimen was then gently pressed transversely across the five inoculum streaks to ensure intimate contact with the agar surface. The plates were incubated at 37o C for 18-24 hours. After the incubation period, the incubated plates were examined for interruption of growth along the streaks of inoculum beneath the specimen and for a clear zone of inhibition beyond its edge. The average width of a Zone of Inhibition (ZOI) along a streak on either side of the test specimen was calculated using Equation 1.

$$W = \frac{(T - D)}{2} \qquad \text{Equation 1}$$

Where:

W: average width of clear zone of inhibition in mm

T: total diameter of test specimen and clear zone in mm

D: diameter of the test specimen in mm

3.2.6.2. Quantitative evaluation

The quantitative evaluation was done using AATCC Test Method 100: Antibacterial Finishes on Textile Materials: Assessment of. The percent reduction of bacteria was calculated using Equation 2:

$$R = \frac{(B - A) * 100}{B} \qquad \text{Equation 2}$$

Where:

R = percent reduction of bacteria

A = the number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated over the desired contact period

B= the number of bacteria recovered from the inoculated untreated test specimen swatches in the jar incubated over the desired contact period

3.2.7. Laundering

The treated fabrics were laundered using AATCC Test Method 61, 3A with modifications to mimic harsher conditions. Laundering was carried out at MLR of 1:10 with 0.5% on weight of fabric of AATCC detergent and 100 steel balls at a temperature of 90^{o} C for 30 minutes. Washing cycle was followed by rinsing in plain water at 40^{o} C for 10 minutes. Finally, the washed swatches were tumble dried. Since a single wash-dry cycle simulated 5 regular wash cycles, the laundering procedure was repeated 2, 5, and 10 times to obtain samples at 10, 25 and 50 wash cycles respectively.

3.2.8. Scanning Electron Microscopy (SEM)

The topography of the control and treated fabrics at zero and fifty wash cycles were visualized and observed under a magnification of 1000 and 5000X using a Scanning Electron Microscope (Model Number JSM-6500F) to determine the differences between the samples.

Sample size of less than 10 mm diameter were prepared by mounting on a stub (small solid cylinder) using a conducting tape and were then kept in desiccators for two days in order to remove excess moisture. The samples were then coated with 20 nm gold using a Hummer VII Sputtering system. Then graphite paint was used along the sample's edge to avoid accumulation of charge. The samples were screwed tight in a sample holder and inserted in the SEM.

SEM scans the surface of the material and projects the image of the material's topography on a computer screen. SEM uses a beam of electrons which hits the surface and generates secondary electron with a low energy of 50EV. The secondary electrons were detected by an Everhart Thornkey detector and final image was projected on a computer screen. The bright spots are caused due to large number of electrons escaping from the surface while dark spots are caused due to the escaping of small number of electrons.

3.2.9. Statistical analysis

Statistical analysis for comparing the antimicrobial properties and durability to laundering of the antimicrobial treated fabrics was done using Statistical Analysis System (SAS version 9.2). The null hypothesis was that there were no statistically significant differences between the antimicrobial properties of the five antimicrobial agents as evaluated via percentage reduction of bacteria after 0, 10, and 50 wash cycles. A three factor design was used to structure the experiment, wherein the independent factors were antimicrobial agents (five levels), wash cycles (three levels), and bacteria (two levels). The durability to laundering performance of antimicrobial agents was statistically compared using analysis of variance (ANOVA) at a 95% confidence level. Outcomes of statistical analysis were augmented with graphs to determine antimicrobial efficiency as a function of the number of wash cycles. Statistical and graphical analyses were expected to:

- Show the trend in the performance of antimicrobial fabrics as a function of wash cycles.
- Rank antimicrobial fabrics in the order of their efficacies at the different wash cycles.

Chapter 4

RESULTS AND DISCUSSION

The polyester-cotton substrates were treated with the five antimicrobial agents, viz., silver, triclosan, QAC, PHMB and chitosan as per the methods described in the previous chapter. Following treatments antimicrobial activity was evaluated qualitatively and quantitatively.

4.1. Qualitative evaluation

The qualitative evaluation was done as per AATCC Test Method 147: Antibacterial Assessment of Textile Materials: Parallel Streak Method. The mean zones of inhibition (ZOI) were calculated for the substrates treated with silver, triclosan and PHMB. The ZOI for QAC and Chitosan substrates were zero since they are bound antimicrobial agents and this method works best for controlled release antimicrobial agents.

4.1.1. Silver

The mean ZOI for polyester-cotton treated with the silver based antimicrobial agent is shown in Table 1 and actual illustrative photographs are displayed in Figure 7.

Table 1: Mean zones of inhibition for Silver treated polyester-cotton blend against *S.aureu*s and *E.coli*

Number of laundry cycles	**Mean zone of inhibition, mm**	
	S.aureus	***E.coli***
0	0	4.0
10	0	0
25	0	0
50	0	0

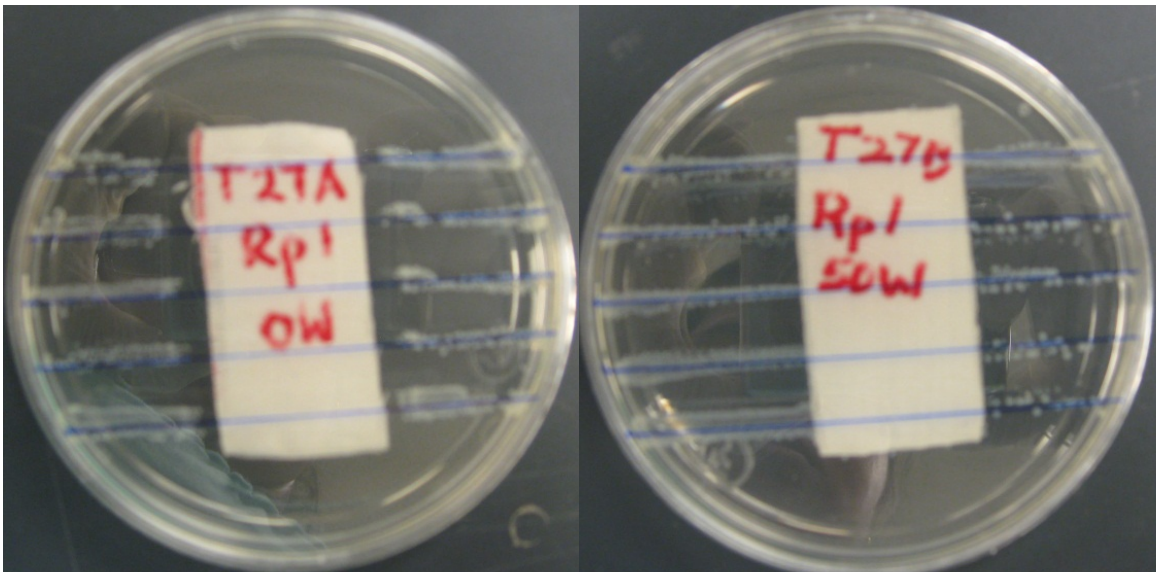

Figure 7: Zone of Inhibition of Silver treated polyester-cotton blend against *E.coli*: after treatment (left) and after 0 laundry cycles (right)

As the data show, silver was not an effective antimicrobial agent against *S.aureus*. Against *E.coli*, silver exhibited a mean ZOI of 4.0 mm (Table 1, Figure 7). For purposes of this study, a ZOI of 2 mm was considered an indication of effective antimicrobial activity, so silver has good antibacterial activity against E.coli. After ten laundry cycles, however, silver lost its effectiveness against E.coli.

4.1.2. Triclosan

Triclosan was found to possess excellent antimicrobial action against both *S.aureus and E.coli* (Table 2; Figures 8 and 9). The mean ZOI of triclosan treated polyester-cotton blend against *S.aureus* after treatment was 22.2 mm and after 50 laundry cycles was 21.1 mm conclusively proving the durability to laundering of the triclosan based antimicrobial. The corresponding values against *E.coli* were 8.5 mm and 7.8 mm again clearly underscoring the excellent and durable antimicrobial properties of triclosan based antimicrobials. The mean ZOI as a function of the number of laundry cycles against both microorganisms is represented graphically in Figure 10.

Table 2: Mean zones of inhibition for Triclosan treated polyester-cotton blend against *S.aureus* and *E.coli*

Number of laundry cycles	**Mean zone of inhibition, mm**	
	S.aureus	***E.coli***
0	22.2	8.5
10	19.8	5.0
25	18.7	6.8
50	21.1	7.8

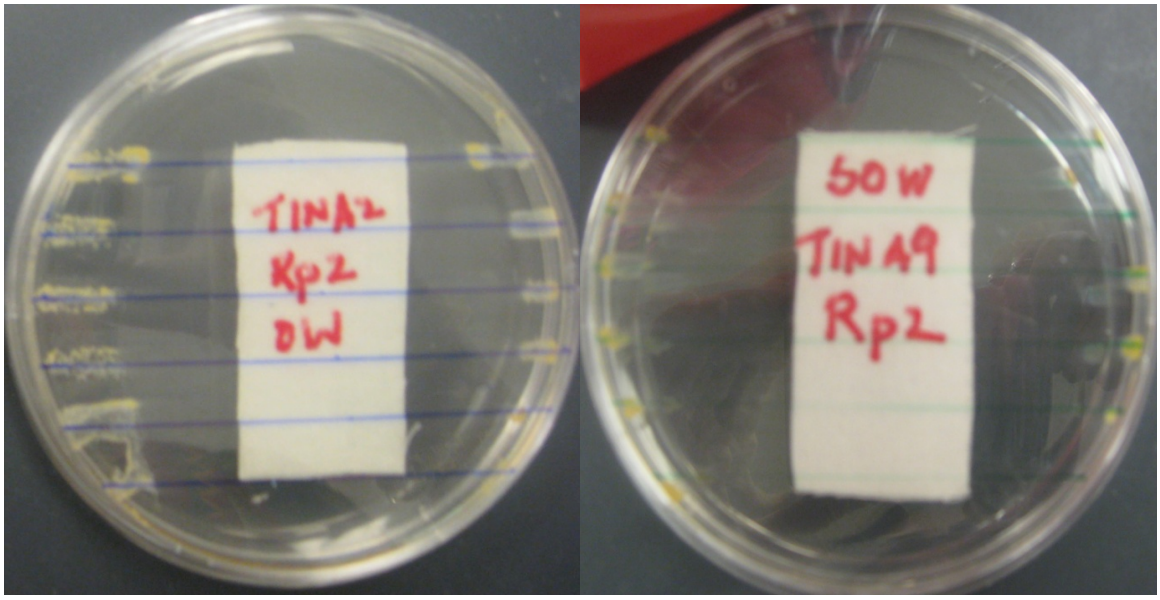

Figure 8: Zone of inhibition of Triclosan treated polyester-cotton blend against *S.aureus*: after treatment (left) and after 50 laundry cycles (right)

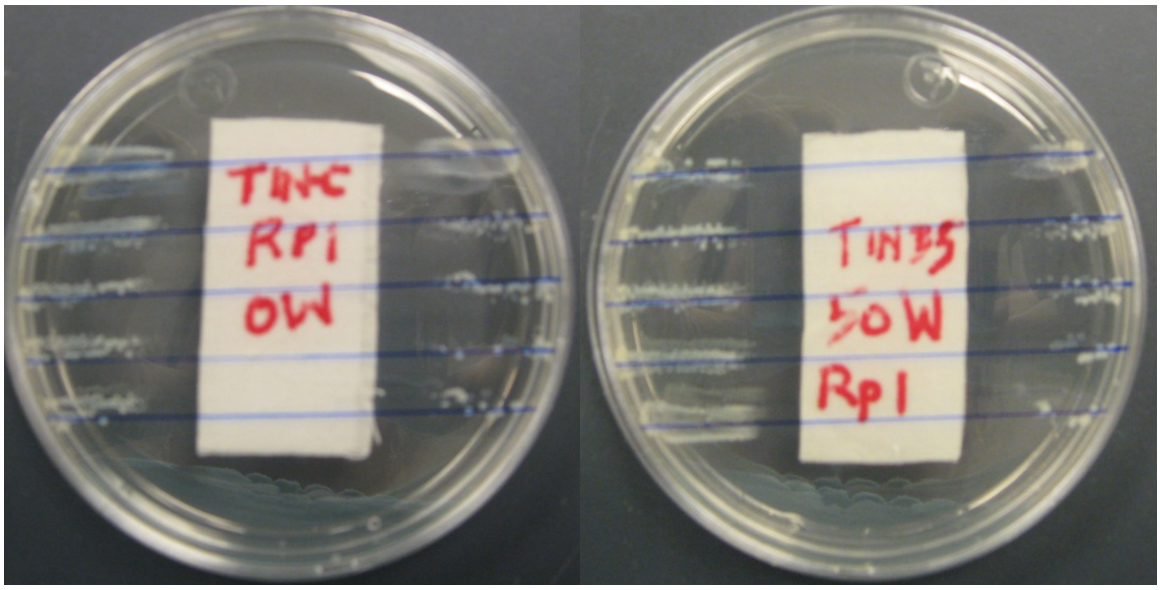

Figure 9: Zone of inhibition of Triclosan treated polyester-cotton blend against *E.coli*: after treatment (left) and after 50 laundry cycles (right)

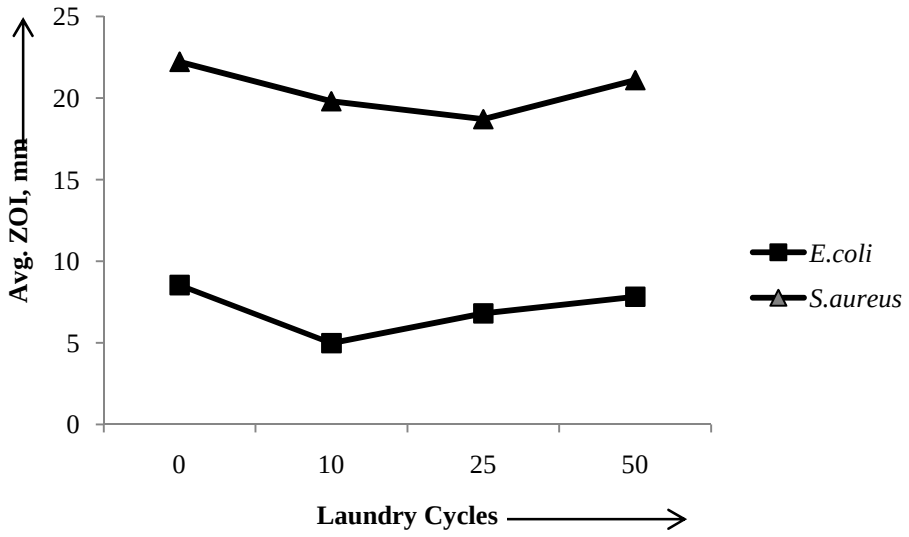

Figure 10: Zone of inhibition of Triclosan treated polyester-cotton blend as a function of number of laundry cycles

4.1.3. Polyhexamethylene Biguanide (PHMB)

For PHMB treated substrates (Table 3; Figures 11, 12, 13) the results were varied. Against *S.aureus*, PHMB treated polyester-cotton blend demonstrated acceptable effectiveness after 50 laundry cycles with a mean ZOI of 3.8 mm. In contrast against *E.coli*, the mean ZOI after treatment was 3.2 mm but which declined dramatically after 10 laundering cycles to 0.3 mm and subsequently to zero mm after 25 wash cycles.

Table 3: Mean zones of inhibition for PHMB treated polyester-cotton blend against *S.aureus* and *E.coli*

Number of laundry cycles	**Mean zone of inhibition, mm**	
	S.aureus	***E.coli***
0	3.8	3.3
10	5.2	0.3
25	6.1	0.0
50	3.8	0.0

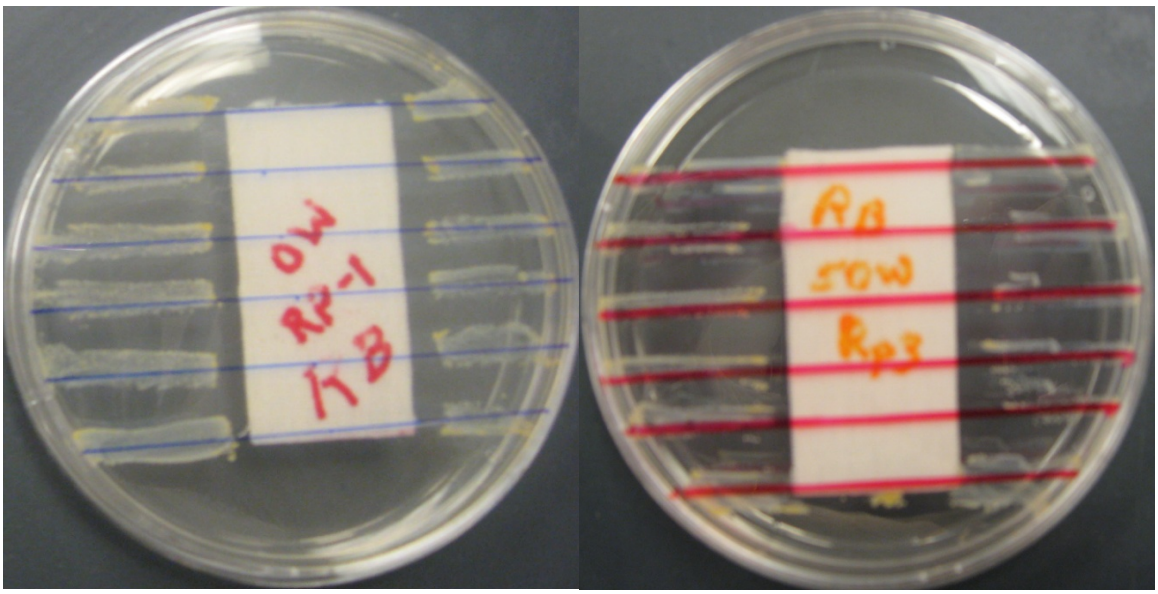

Figure 11: Zone of inhibition of PHMB treated polyester-cotton blend against *S.aureus*: after treatment (left) and after 50 laundry cycles (right)

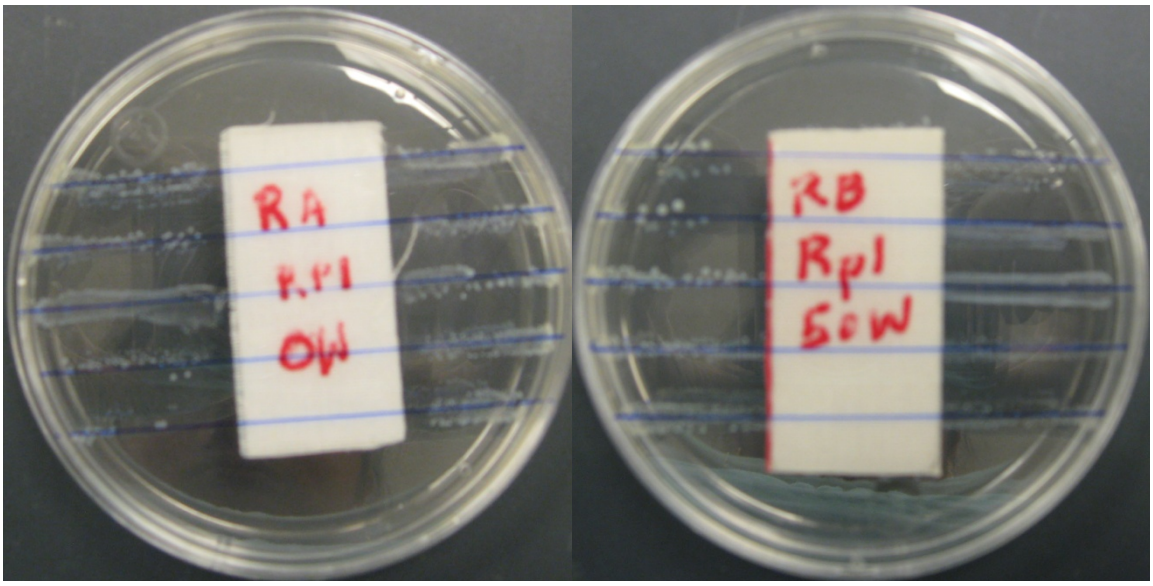

Figure 12: Zone of inhibition of PHMB treated polyester-cotton blend against *E.coli*: after treatment (left) and after 50 laundry cycles (right)

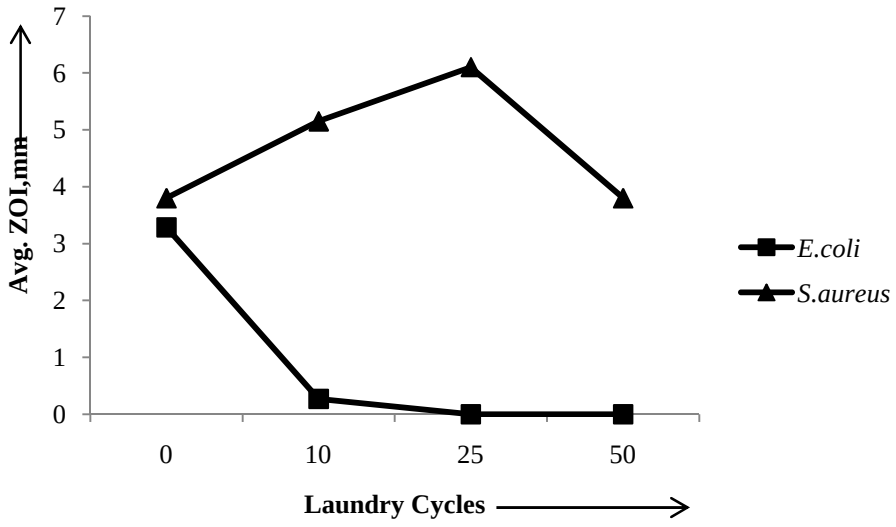

Figure 13: Zone of inhibition of PHMB treated polyester-cotton blend as a function of number of laundry cycles

4.1.4. Summary of durability to laundering of antimicrobial agents (qualitative evaluation)

The comparative durability to laundering of silver, PHMB, and triclosan are illustrated in Figures 14 and 15 against *S.aureus* and *E.coli* respectively. The summary graphs buttress the fact that the triclosan based antimicrobial agent has superior durability to laundering than the other controlled release antimicrobial agents in this study.

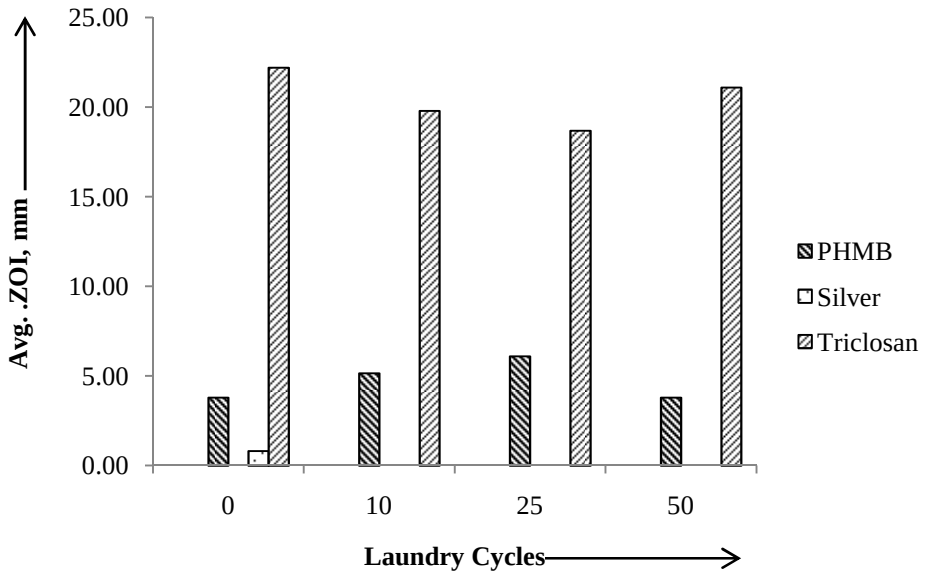

Figure 14: Durability to laundering of Silver, Triclosan and PHMB treated polyester-cotton blend against *S.aureus* (qualitative evaluation)

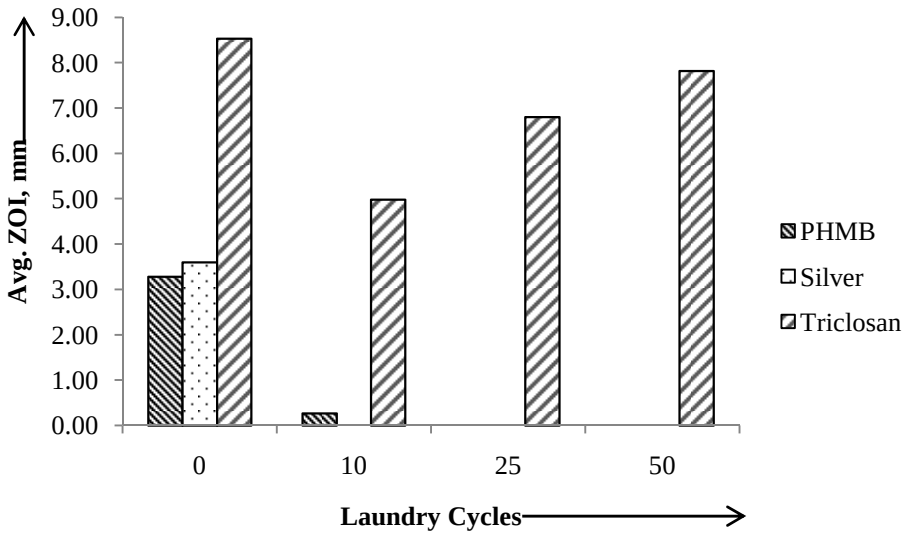

Figure 15: Durabilty to laundering of Silver, Triclosan and PHMB treated polyester-cotton blend against *E.coli* (qualitative evaluation)

4.2.Quantitative evaluation

Quantitative evaluation was carried out as per AATCC Test Method 100: Antibacterial Finishes on Textile Materials: Assessment of. All data reported are the means of three replications.

4.2.1. Silver

Table 4 reports the results for the silver based antimicrobial agent. After treatment ("0" wash cycles) silver was effective against both *S.aureus* and *E.coli* with a reduction percentage of 100 and 99.6% respectively.

Table 4: Percentage reduction of bacteria for Silver treated polyester-cotton blend against *S.aureus* and *E.coli*

Number of laundry cycles	**Reduction of bacteria, %**	
	S.aureus	***E.coli***
0	100.0	99.6
10	100.0	63.7
50	95.5	67.7

After 10 wash cycles, antimicrobial activity against *S.aureus* remained at 100% reduction dropping to 95.5% after 50 wash cycles. However, the activity against *E.coli* dramatically reduced to 67.7% after 50 laundry cycles. The percentage reduction of bacteria as a function of wash cycles is shown in Figure 16.

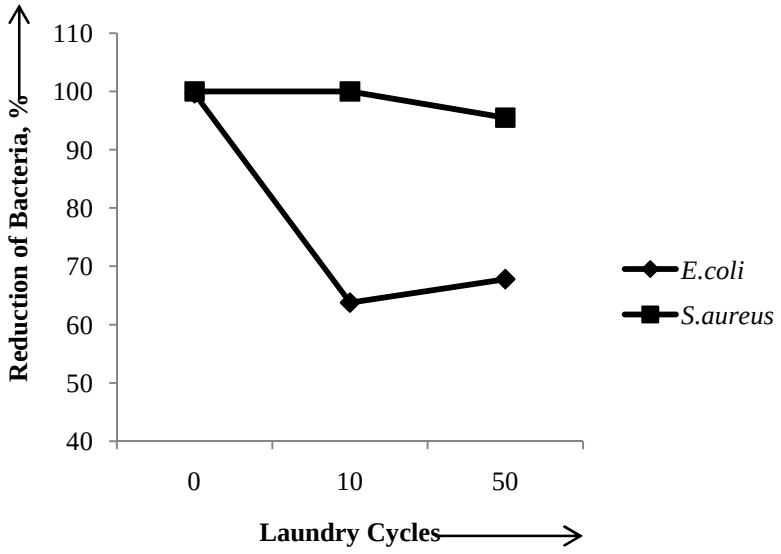

Figure 16: Percentage reduction of bacteria of silver treated polyester-cotton blend as a function of number of laundry cycles

4.2.2. Triclosan

The data for triclosan (Table 5) are consistent with qualitative evaluation results in that triclosan was extremely effective against both bacteria and the efficacy did not diminish after 50 wash cycles.

Table 5: Percentage reduction of bacteria for Triclosan treated polyester-cotton blend against *S.aureus* and *E.coli*

Number of laundry cycles	**Reduction of bacteria, %**	
	S.aureus	***E.coli***
0	100.0	100.0
10	100.0	100.0
50	100.0	100.0

4.2.3. QAC

At "0" wash cycles, QAC possessed excellent antimicrobial activity against *S.aureus* with 100% reduction as well as against *E.coli* with a reduction of 97% (Table 6). The performance after laundering however was considerably different against the two bacteria. Against *S.aureus* the efficacy gradually decreased with increasing number of wash cycles and percentage reduction was 82.5% after 50 wash cycles. Against *E.coli* the reduction in efficacy was steep and only 48.5% after 50 wash cycles (Figure 17).

Table 6: Percentage reduction of bacteria for QAC treated polyester-cotton blend against *S.aureus* and *E.coli*

Number of laundry cycles	**Reduction of bacteria, %**	
	S.aureus	***E.coli***
0	100.0	97.0
10	97.2	38.6
50	82.6	48.5

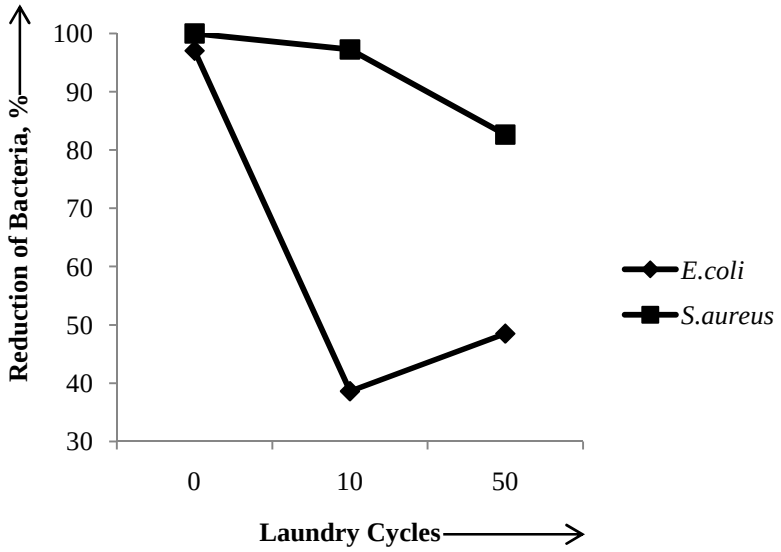

Figure 17: Percentage reduction of bacteria of QAC treated polyester-cotton blend as a function of number of laundry cycles

4.2.4. PHMB

PHMB was equally effective against *E.coli* and *S.aureus* at 0^{th} wash cycle with 99.7% and 100% reduction respectively (Table 7). On laundering the effectiveness against the two bacteria were again markedly different (Figure 18). At 50 wash cycles, the percentage reduction of *S.aureus* was 78.6% but against *E.coli* it was 52.3%.

Table 7: Percentage Reduction of bacteria for PHMB treated polyester-cotton blend against *S.aureus* and *E.coli*

Number of laundry cycles	**Reduction of bacteria, %**	
	S.aureus	***E.coli***
0	100.0	99.7
10	100.0	95.1
50	78.6	52.3

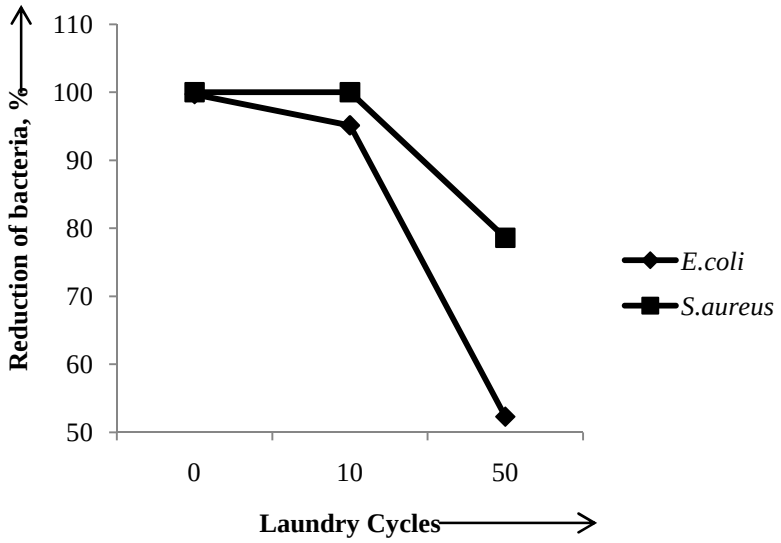

Figure 18: Percentage reduction of bacteria of PHMB treated polyester-cotton blend as a function of number of laundry cycles

4.2.5. Chitosan

Antimicrobial activity of chitosan against *E.coli* and *S.aureus* after treatment ("0" wash cycle) was excellent with 99.6% and 100% reduction of bacteria respectively. The activity against *S.aureus* remained high decreasing only to 92% after 50 wash cycles. The efficacy against *E.coli* however reduced significantly to 51% after 50 wash cycles (Figure 19).

Table 8: Percentage Reduction of bacteria for chitosan treated polyester-cotton blend against *S.aureus* and *E.coli*

Number of laundry cycles	**Reduction of bacteria, %**	
	S.aureus	***E.coli***
0	100.0	99.6
10	99.3	33.8
50	92.0	51.0

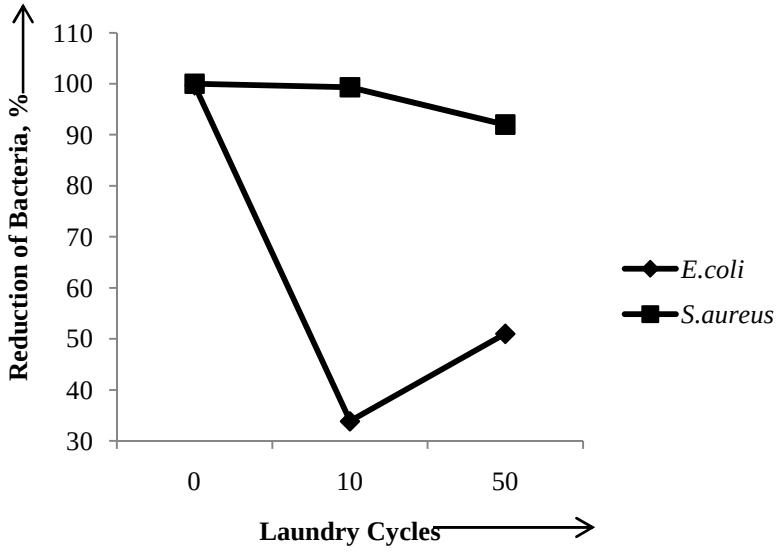

Figure 19: Percentage reduction of bacteria of chitosan treated polyester-cotton blend as a function of number of laundry cycles

Summarizing the antimicrobial efficacy of the individual agents against both bacteria, it is observed that triclosan was most effective against both *E.coli* and *S.aureus* after 50 wash cycles. Silver, QAC, PHMB and chitosan had higher efficacy against *S.aureus*, a gram positive bacteria than against *E.coli*, a gram negative bacteria. A probable explanation could be that the stronger outer cell wall of gram negative bacteria such as *E.coli* restricts the antimicrobial molecules from penetrating and killing the bacteria efficiently. A second observation was the antimicrobial action of Silver, QAC, PHMB and chitosan decreased with increase in number of laundry cycles and the decrease was more pronounced against *E.coli*.

4.3.Scanning Electron Microscopy

SEM analysis of the treated and laundered substrates at 10, and 50 wash cycles revealed no discernible differences in the topography of the specimen samples (Figure 20, 21, and 22).

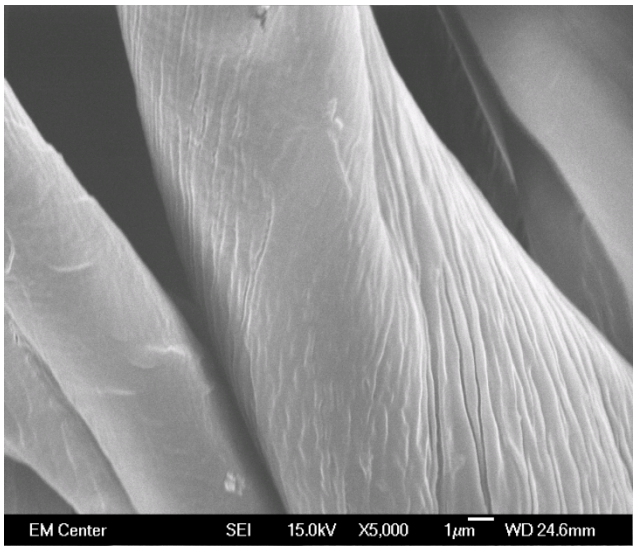

Figure 20: SEM Image of untreated fabric

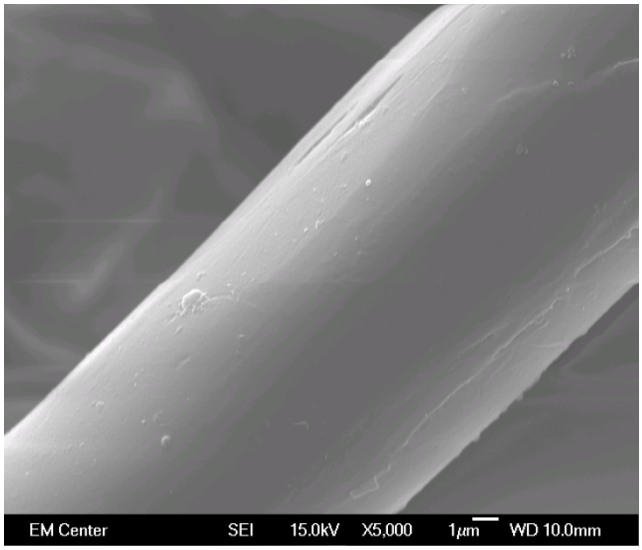

Figure 21: SEM Image of Triclosan treated fabric

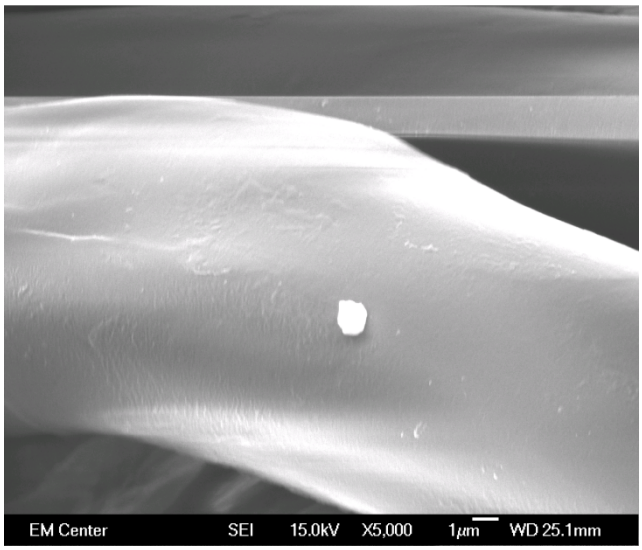

Figure 22: SEM Image of Triclosan treated fabric after 50 wash cycles

4.4.Statistical analysis of the durability to laundering of antimicrobial agents

Quantitative results (Table 9) were statistically analyzed using ANOVA at a 95% confidence interval using SAS Version 9.2.

Table 9: Percentage reduction of bacteria by antimicrobial agents at the distinct wash cycles

	E.coli			***S.aureus***		
Wash Cycles-> / ***Agents***	**0**	**10**	**50**	**0**	**10**	**50**
Chitosan	99.6	33.8	51.0	100.0	99.3	92.0
PHMB	99.7	95.1	52.3	100.0	100.0	78.6
QAC	97.0	38.6	48.5	100.0	97.2	82.6
Silver	99.6	63.7	67.7	100.0	100.0	95.5
Triclosan	100.0	100.0	100.0	100.0	100.0	100.0

At the 0^{th} wash cycle (after treatment but before laundering) the efficacy of Silver, Triclosan, PHMB and Chitosan against *E. coli* were not significantly different from each other (Table 10). Only QAC with 97% reduction of *E. coli* was statistically significantly different compared with the other antimicrobial agents ($P<0.0001$). It should be noted, however, that a 97% reduction of bacteria is sufficiently high to warrant the use of QAC based agents for most antimicrobial applications. Against *S.aureus*, all agents exhibited 100% reduction of bacteria at the 0th wash cycle. Figure 23 is a schematic representation of comparative efficacy of the antimicrobial agents at the 0^{th} wash cycle against *E. coli* and *S.aureus*.

Table 10: P values at 0th wash cycle against *E.coli*

Agent 1	Agent 2	P value
Silver	Triclosan	0.19
Silver	PHMB	0.73
Silver	Chitosan	0.89
Triclosan	PHMB	0.28
Triclosan	Chitosan	0.35
PHMB	Chitosan	0.91
Silver	QAC	<0.0001
Triclosan	QAC	<0.0001
PHMB	QAC	<0.0001
Chitosan	QAC	<0.0001

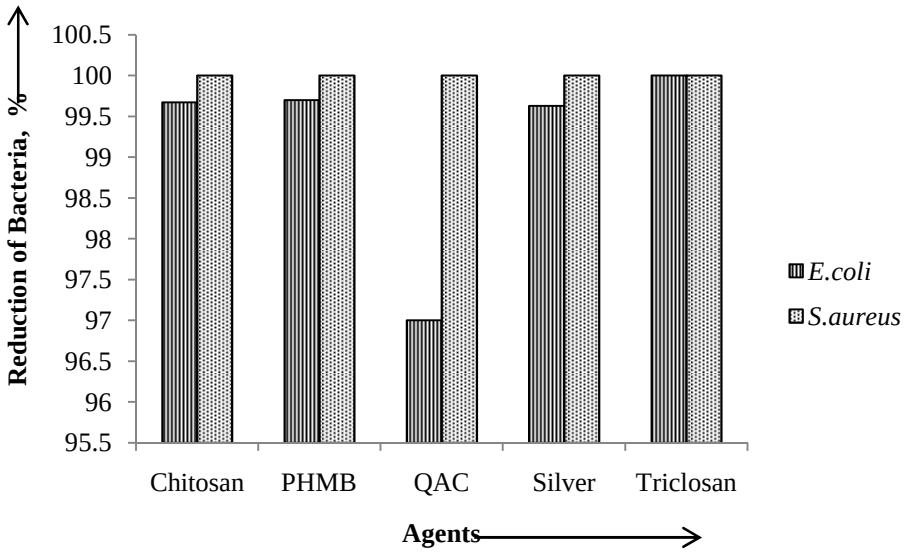

Figure 23: Percentage reduction of bacteria by antimicrobial agents at 0th wash cycle against *E.coli* and *S.aureus*

After 10 wash cycles (Table 11; Figure 24) PHMB and triclosan maintained their efficacies at 95.1% and 100% against *E.coli* respectively and were not significantly different from each other (P=0.57). The efficacy of silver reduced to 63.7% against *E.coli* which is statistically significantly different from triclosan (P=0.0002) and PHMB (<0.0001). Similarly, the performance of the QAC based antimicrobial and chitosan reduced to 38.6% and 33.8% respectively against *E.coli*

which were also statistically different from each other with P values <0.0001(Table 11). Against *S. aureus*; silver, PHMB and triclosan exhibited 100% efficacy after 10 wash cycles. QAC and chitosan exhibited 97.2% and 99.3% reduction of bacteria which is not statistically significant from triclosan with P=0.05, and P=0.61 respectively (Table 12; Figure 24).

Table 11: P values after 10 wash cycles against *S.aureus*

Agent 1	Agent 2	P value
Silver	Triclosan	1
Silver	PHMB	0.05
Silver	Chitosan	0.61
Triclosan	PHMB	1
Triclosan	Chitosan	0.61
PHMB	Chitosan	0.61
Silver	QAC	0.05
Triclosan	QAC	0.05
PHMB	QAC	0.05
Chitosan	QAC	0.085

Table 12: P values after 10 wash cycles against *E.coli*

Agent 1	Agent 2	P value
Silver	Triclosan	0.0002
Silver	PHMB	<0.0001
Silver	Chitosan	<0.0001
Triclosan	PHMB	0.57
Triclosan	Chitosan	<0.0001
PHMB	Chitosan	<0.0001
Silver	QAC	0.0004
Triclosan	QAC	<0.0001
PHMB	QAC	<0.0001
Chitosan	QAC	<0.0001

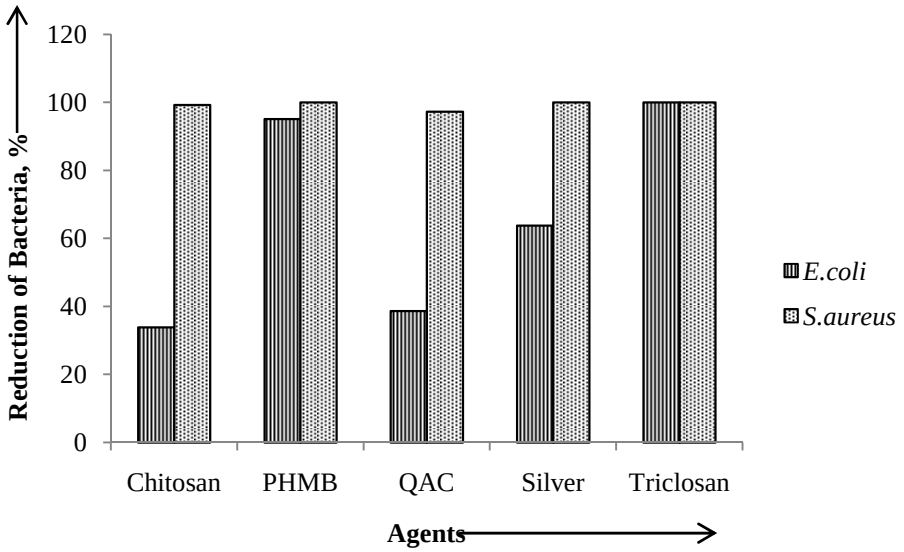

Figure 24: Percentage reduction of bacteria by antimicrobial agents at 10th wash cycle against *E.coli* and *S.aureus*

The performance of triclosan after 50 wash cycles was 100% reduction of *E.coli* which was statistically significantly different from all other agents. (Table 13; Figure 25). Silver exhibited a percentage reduction of 67.7% followed by PHMB with 52.3% (Table 9) which were statistically not significantly different from each other with P value of 0.34 (Table 13). The performance of chitosan was different from silver (P=0.04) but not from PHMB (P=0.88) and QAC (P=0.76). The performance of QAC was significantly different from silver (P=0.006) but not different from PHMB (P=0.59). Against *S.aureus*, the performance of silver and chitosan were not significantly different from triclosan with P values of 0.59 and 0.29 respectively. However, PHMB and QAC were statistically significantly different from silver, chitosan and triclosan (Table 14; Figure 25).

Table 13: P values after 50 wash cycles against *E.coli*

Agent 1	Agent 2	P value
Silver	Triclosan	0.0011
Silver	PHMB	0.34
Silver	Chitosan	0.04
Triclosan	PHMB	<0.0001
Triclosan	Chitosan	<0.0001
PHMB	Chitosan	0.88
Silver	QAC	0.006
Triclosan	QAC	<0.0001
PHMB	QAC	0.59
Chitosan	QAC	0.76

Table 14: P values after 50 wash cycles against *S.aureus*

Agent 1	Agent 2	P value
Silver	Triclosan	0.59
Silver	PHMB	0.0052
Silver	Chitosan	0.55
Triclosan	PHMB	0.007
Triclosan	Chitosan	0.29
PHMB	Chitosan	0.006
Silver	QAC	0.033
Triclosan	QAC	0.03
PHMB	QAC	0.37
Chitosan	QAC	0.06

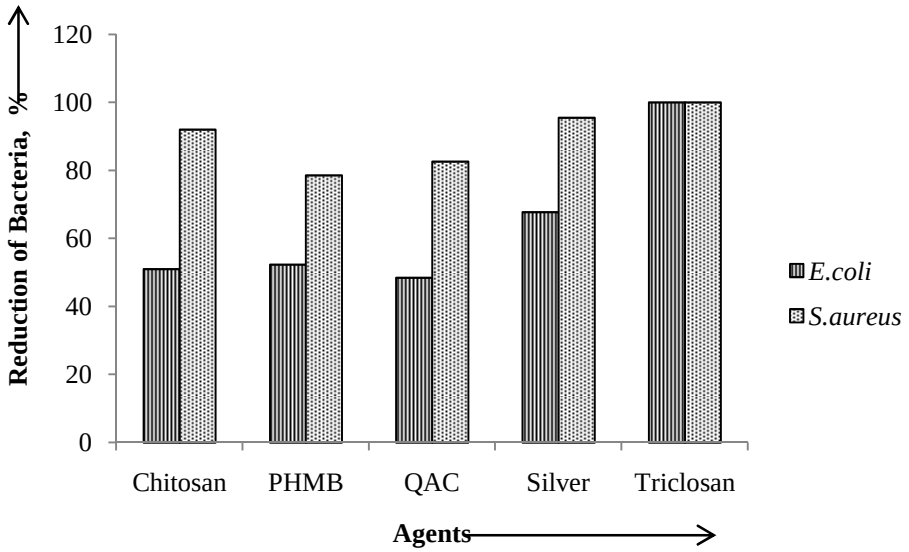

Figure 25: Percentage reduction of bacteria by antimicrobial agents at 50th wash cycle against *E.coli* and *S.aureus*

Figures 26 and 27 illustrate and summarize the comparative durability to laundering property of the five antimicrobial agents in this study. It can be reiterated that after treatment all selected agents possessed excellent antimicrobial activity against *E.coli*. With each wash cycle, the antimicrobial properties gradually abated for all agents with the exception of Triclosan. Against *S.aureus* all antimicrobial agents performed comparably after treatment i.e. 0th wash cycle. However, as opposed to their behavior against *E.coli*, three agents viz. Triclosan, Silver, Chitosan retained high antimicrobial activity against *S.aureus* after 50 wash cycles. The PHMB and QAC based antimicrobial agents did lose activity against *S.aureus* but not to the extent as the loss of activity against *E.coli*.

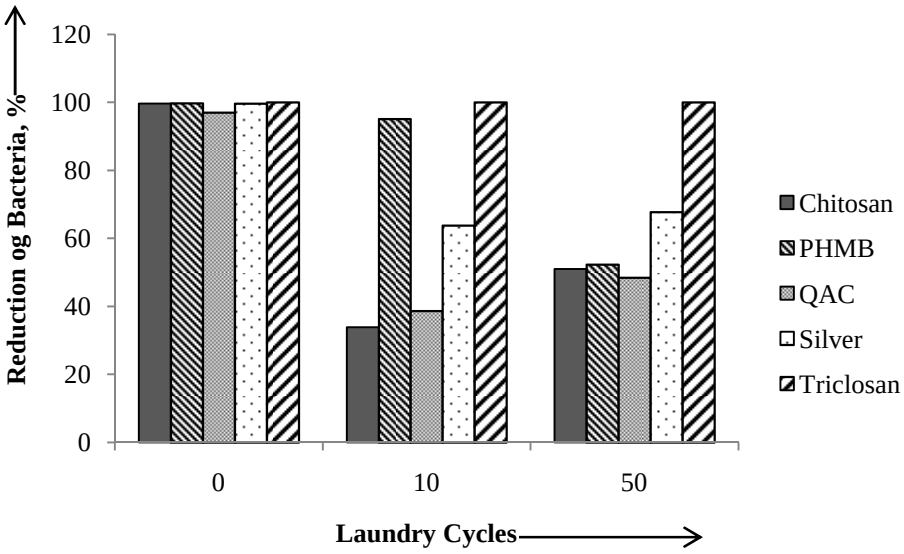

Figure 26: Comparative durability to laundering of antimicrobial agents against *E.coli*

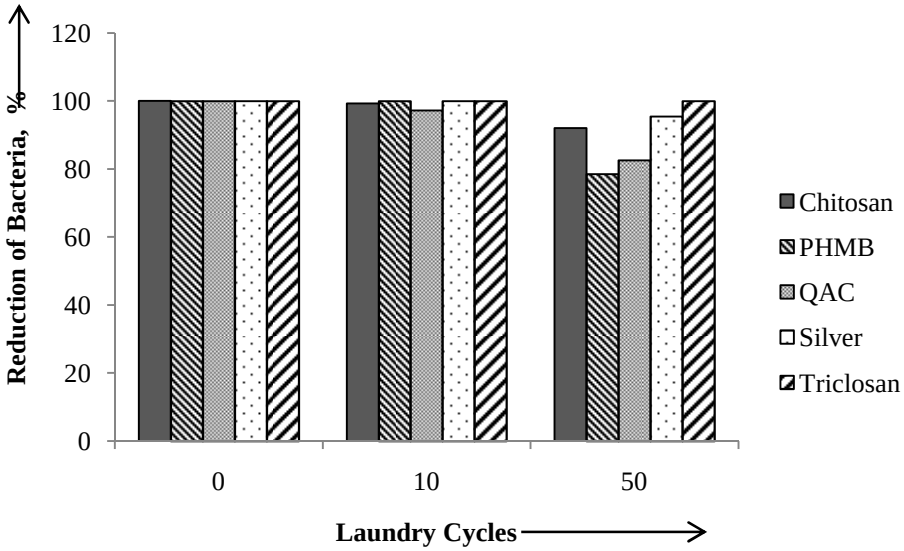

Figure 27: Comparative durability to laundering of antimicrobial agents against *S.aureus*

Chapter 5

CONCLUSIONS

This research investigated the efficacy and durability to laundering of five antimicrobial agents with distinctive chemistries and diverse modes of action on a polyester/cotton blend hospital textile. The five antimicrobial agents comprised different antimicrobial entities; silver, triclosan, QAC, PHMB and chitosan. Antimicrobial activity and durability were evaluated against two microbes; *S.aureus*, a gram positive bacteria and *E.coli*, a gram negative bacteria. Data obtained were statistically analyzed at a 95% confidence level to test the following null hypotheses:

1. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *S.aureus* at "0" wash cycles (before laundering).

   All the antimicrobial agents under investigation exhibited 100% reduction of S.aureus. Null hypothesis is ***accepted.***

2. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *E.coli* at "0" wash cycles (before laundering).

   The null hypothesis is ***rejected*** *when comparing the performance of QAC with all other antimicrobial agents since QAC exhibited a lower percentage reduction of bacteria. However, the null hypothesis is accepted when comparing the performance of silver, triclosan, PHMB and chitosan since statistically no significant difference in effectiveness were found between these agents.*

3. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *S.aureus* after 10 wash cycles.

   The comparative P value against S.aureus for different agents after 10 wash cycles were greater than 0.05 and the null hypothesis is ***accepted****.*

4. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *E.coli* after 10 wash cycles.

   The null hypothesis is ***accepted*** *when triclosan and PHMB are compared as the P value is 0.57. The null hypothesis is* ***rejected*** *when silver, triclosan, QAC, PHMB, and chitosan are compared (P value <0.05).*

5. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *S.aureus* after 50 wash cycles.

   The null hypothesis is ***accepted*** *when silver is compared with triclosan and chitosan. However, the null hypothesis is* ***rejected*** *when silver, triclosan and chitosan are compared with PHMB, and QAC. Therefore, the agents can be sorted in terms of their effectiveness against S.aureus after 50 wash cycles as follows:*

   Triclosan = Silver = Chitosan > PHMB = QAC

6. There is no statistically significant difference between the effectiveness of the different antimicrobial agents against *E.coli* after 50 wash cycles.

   The null hypothesis is ***rejected*** *when triclosan is compared with the other antimicrobial agents. The agents can be sorted in terms of their effectiveness against E.coli after 50 wash cycles as follows:*

   Triclosan > Silver > PHMB > chitosan = QAC

Limitations of present study and recommendations for future study

This study was limited to one textile substrate and two microorganisms. Also, the AATCC Test Method 100 indicates a variation of 8% within analyst and about 18% between analysts in a given laboratory. It is also noted that laboratory conditions wherein a textile fabric is challenged with a known quantity of a pre-determined organism does not necessarily replicate conditions in a healthcare environment where textile substrates may be simultaneously subjected to exposure by several organisms. Additionally, the two evaluation methods used in this study are among a number of other evaluation procedures some of which may yield dissimilar results depending on the type of antimicrobial agent i.e. controlled release or bound and the compatibility of the test method. As such, this study cannot make comprehensive overviews and the results are specific to the polyester/cotton substrate and the two organisms examined in the study. Future work can be expanded to include multiple substrates and other challenge organisms especially with regard to studying a different gram-negative bacterium such as *Klebsiella pneumonia*. A study of antimicrobial activity kinetics may also be useful in determining the rate of kill of the microorganisms. A third recommendation for future study is to investigate the efficacy and durability of textile substrates treated with permutations and combinations of the antimicrobial agents in order to determine their synergistic activity. Finally, a cost-benefit analysis and a study of the environmental ramifications of the antimicrobial agents will need to be conducted to arrive at a definitive conclusion regarding the best antimicrobial agent and/or agents for hospital textiles.

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