Research Article

Evaluation of Durability to Laundering of Triclosan and Chitosan on a Textile Substrate

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In a hospital environment, textile substrates have been implicated as a vector in the transmission of disease. To mitigate the harmful spread of disease via textile substrates, an effective measure is treatment of textiles with antimicrobial agents. The current investigation compares one of the most widely used chemically synthesized antimicrobial agents, triclosan with chitosan, a naturally occurring antimicrobial agent. For the study, samples of a common polyester/cotton textile used in hospital settings were treated with triclosan and chitosan based antimicrobial agents. Following treatment, the samples were analyzed for their effectiveness against *Staphylococcus aureus* and *Escherichia coli* using standard AATCC Test Methods. The efficacy of the treatment to laundering was then evaluated by subjecting the treated samples to 50 washings and repeating the tests against the challenge microorganisms. Data obtained were statistically analyzed at a 95% confidence interval. Results showed that before laundering both triclosan and chitosan treated samples were extremely effective as antimicrobial agents. After laundering, chitosan was less effective against *E. coli* but maintained efficacy against *S. aureus*. The effectiveness of triclosan was not adversely affected after the laundering treatments.

1. Introduction

The Centers for Disease Control and Prevention, USA, estimate that approximately 1.7 million healthcare associated infections (HAIs) and 98,987 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 [1]. Textile substrates have been implicated as one of the vectors of transmission of infections. Fortuitously, the use of antimicrobial textiles is an effective deterrent against the spread of infection-causing bacteria [2].

Triclosan, a chlorinated phenolic compound, is a derivative of a diphenyl ether compound. Triclosan is most widely used in various medical and consumer care products. Triclosan belongs to *leaching* or controlled release category of antimicrobial agents [2, 3]. The mechanism of action of triclosan on a textile substrate is illustrated in Figure 1. The figure depicts a treated fiber containing a reservoir of antimicrobial agent. When the chemical agent ions are

released outside the fiber they destroy microbes present in the vicinity. Triclosan inhibits the growth of microbes by using an electrochemical mode of action to penetrate and disrupt the cell wall of microbes [2, 4]. It is probable that, with eventual usage and repetitive laundering of the treated material, the reservoir in the fiber will get depleted and lose its antimicrobial efficacy [5]. As well, via laundings the ions may enter the water stream. Triclosan has been reported to cause environmental problems due to its nonbiodegradability. Other studies have reported increased resistance to triclosan by certain strains of *S. aureus* and other bacteria. The U.S. Food and Drug Administration is currently engaged in an ongoing scientific and regulatory review of triclosan [6].

Chitin, a poly(β -(1-4)-N-acetyl-D-glucosamine), is a natural polysaccharide. 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%, it is called chitosan [7]. Chitosan belongs to the category of *nonleaching* or the bound type antimicrobial agents, wherein the antimicrobial agent is fixed to the fiber surface via

FIGURE 1: Mechanism of action of triclosan.

FIGURE 2: Mechanism of action of chitosan.

covalent bonds and is able to destroy the microbes that come in contact with the fiber [2]. The mechanism of action of chitosan on a textile substrate is shown in Figure 2. 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 [8, 9]. Other studies suggest that the formation of the polymeric substance around the bacterial cell prevents the nutrients from entering the cell [10].

Based on a literature review of 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 purpose 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. Therefore, the endeavor of the present research was to evaluate the durability to laundering of two such agents, triclosan and chitosan. Secondly, as mentioned before, there are rising concerns with regard to triclosan, a chemically synthesized agent. The study would consequently also shed

light on whether chitosan, a naturally derived antimicrobial agent, could serve as a potential replacement for synthetic antimicrobial agents.

2. Materials and Methods

2.1. Materials. The antimicrobial agent based on triclosan was a halogenated phenol with the chemical constitution 5-chloro-2-(2,4-dichlorophenoxy) phenol. A 6% chitosan based formulation was the other antimicrobial agent. The plain weave fabric treated with the two antimicrobial agents had the following characteristics: fiber content: 65% polyester/35% cotton; weight: 154 g/m² (ASTM D3776); fabric count: 158; and thickness: 0.017 inches (ASTM D1777-96). The treated substrates were challenged against *Staphylococcus aureus* (ATCC 6538, PML Microbiologicals, Inc.), Gram positive bacteria, and *Escherichia coli* (ATCC 8739, PML Microbiologicals, Inc.), Gram negative bacteria.

2.2. Methods. The purpose of the study was to compare the antimicrobial efficacy of triclosan and chitosan when applied to a substrate using manufacturers' recommended

concentration and treatment protocols. Adhering to the recommendations ensured optimum performance of the two agents. Hence, the treatment methods for triclosan and chitosan are different as enumerated below.

2.2.1. Triclosan Treatment Method. 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% on weight of fabric (owf) with a material to liquor ratio (MLR) of 1:50. As per the MLR calculations, required amount of water was measured in a treatment bath and heated to 50°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°C. Treatment was continued at this temperature for 60 minutes. Finally fabrics were rinsed and air-dried.

2.2.2. Chitosan Treatment Method. 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 4.5 and 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°C in the curing chamber for 5 minutes.

2.2.3. Laundering Procedure. The treated samples were laundered using AATCC Test Method 61, 3A with modifications to mimic harsher industrial laundry conditions. Launderings were carried out using MLR of 1:10 with 0.5% on weight of fabric of AATCC detergent and 100 steel balls at a temperature of 90°C for 30 minutes. Washing cycle was followed by rinsing in plain water at 40°C for 10 minutes followed by tumble drying. Since a single wash-dry cycle simulated 5 regular wash cycles, the laundering procedure was repeated 10 times to obtain samples after 50 wash cycles.

2.2.4. Evaluation Methods. Treated and washed samples were challenged with strains of *S. aureus* and *E. coli*. The antibacterial activity was evaluated using AATCC Test Method 147: Antibacterial Assessment of Textile Materials: Parallel Streak Method and AATCC Test Method 100: Assessment of Antibacterial Finishes on Textile Materials.

For AATCC Test Method 147, specimen sizes of 25 × 50 mm were used. A loop full of the respective culture was transferred to the surface of a 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 streaks to ensure intimate contact with the agar surface. The plates were incubated at 37°C for 18–24 hours and 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

$$W = \frac{(T - D)}{2}, \quad (1)$$

where W is the average width of clear zone of inhibition in mm, T is the total diameter of test specimen and clear zone in mm, and D is the diameter of the test specimen in mm.

For AATCC Test Method 100, three discs of treated and untreated samples measuring 48 mm were inoculated with 1 mL of inoculum ($1-2 \times 10^5$ organisms) in two separate 100 mL jars. The jars were incubated for 24 hours at 37°C. The samples were then neutralized with 100 mL of Tween 80 solution (Sigma-Aldrich) and were vigorously shaken for 1 minute. The solution was then eluted and plated with appropriate dilution in order to obtain countable colonies. The percent reduction of bacteria was calculated using

$$R = \frac{(B - A) * 100}{B}, \quad (2)$$

where R is the percent reduction of bacteria, A is the number of bacteria recovered from the inoculated treated test samples in the jar incubated over the desired contact period, and B is the number of bacteria recovered from the inoculated untreated test samples in the jar incubated over the desired contact period.

2.2.5. Statistical Analysis. The antimicrobial properties and durability to laundering of the antimicrobial treated fabrics were compared using Statistical Analysis System (SAS version 9.2, Cary, NC). The null hypothesis was that there were no statistically significant differences between triclosan and chitosan on a textile substrate. A three-factor design was used to structure the experiment, wherein the independent factors were antimicrobial agents, wash cycles, and bacteria. The treatment procedure, laundering, and antimicrobial testing of samples were replicated three times. The statistical evaluation was done using analysis of variance (ANOVA) at a 95% confidence level.

3. Results and Discussion

The mean zone of inhibition (ZOI, mm) as calculated using AATCC Test Method 147 for the polyester/cotton treated with triclosan and chitosan is shown in Table 1. Actual illustrative photographs against *E. coli* are displayed in Figures 3 and 4.

For purposes of this study, a mean ZOI of 2 mm was considered an indication of effective antimicrobial activity. Triclosan was found to possess excellent antimicrobial action against both *S. aureus* and *E. coli* (Table 1 and Figure 3). The mean ZOI against *S. aureus* before laundering the treated substrate was 22.2 mm and after 50 laundry cycles there was a slight decrease to 21.1 mm conclusively proving

FIGURE 3: Zone of inhibition of triclosan treated polyester/cotton blend against *E. coli*: after treatment (a) and after 50 laundry cycles (b).

FIGURE 4: Zone of inhibition of chitosan treated polyester/cotton blend against *E. coli*: after treatment (a) and after 50 laundry cycles (b).

TABLE 1: Mean zone of inhibition (mm) for treated polyester/cotton substrate against *S. aureus* and *E. coli* after treatment (0 laundry cycles) and after 50 laundry cycles.

Antimicrobial agent	<i>S. aureus</i> ZOI (mm)		<i>E. coli</i> ZOI (mm)	
	Laundry cycles			
	0	50	0	50
Triclosan	22.2	21.1	8.5	7.8
Chitosan	0.0	0.0	0.0	0.0

the durability to laundering of the samples treated with triclosan. The values against *E. coli* were 8.5 mm before laundering and 7.8 mm after 50 laundry cycles again clearly underscoring the excellent and durable antimicrobial properties of triclosan based antimicrobials. The mean ZOI for chitosan treated substrates was 0 mm against both types of bacteria (Table 1 and Figure 4). This result is logical because AATCC Test Method 147 works best with *leaching* type of

TABLE 2: Percentage reduction in bacteria of treated polyester/cotton substrate against *S. aureus* and *E. coli* after treatment (0 laundry cycles) and after 50 laundry cycles.

Antimicrobial agent	<i>S. aureus</i> (% reduction)		<i>E. coli</i> (% reduction)	
	Laundry cycles			
	0	50	0	50
Triclosan	100.0	100.0	100.0	100.0
Chitosan	100.0	92.0	99.6	51.0

antimicrobials and as explained previously chitosan belongs to the bound or *nonleaching* type of antimicrobials. The non-leaching characteristic of chitosan was further emphasized by the absence of bacteria on the underside of the fabrics.

Table 2 reports the results obtained using AATCC Test Method 100. The data for triclosan are consistent with the results obtained using AATCC Test Method 147. Triclosan was extremely effective against both types of bacteria and

TABLE 3: Statistical comparison (P values) of polyester/cotton substrate against *S. aureus* and *E. coli* after treatment (0 laundry cycles) and after 50 laundry cycles.

Antimicrobial agent	<i>S. aureus</i>		<i>E. coli</i>	
	Laundry cycles			
	0	50	0	50
Triclosan (agent 1)	1	0.29	0.35	<0.0001
Chitosan (agent 2)				

the efficacy did not diminish after 50 launderings. Antimicrobial activity of chitosan against *S. aureus* and *E. coli* and after treatment was excellent as well with 100% and 99.6% reduction of bacteria, respectively. After 50 laundering cycles, the activity against *S. aureus* remained high and only slightly decreased to 92%. However, the efficacy of chitosan against *E. coli* reduced significantly to 51% after 50 launderings. A probable explanation is 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.

Statistical analysis (Table 3) showed that the efficacies of unlaundered samples ("0" wash cycles) of substrates treated with triclosan and chitosan against *E. coli* were not statistically different from each other (P value = 0.35). Similarly, against *S. aureus* both the agents exhibited 100% reduction of bacteria and therefore $P = 1$. After 50 launderings, the performance of chitosan against *E. coli* was reduced to 51% which was statistically significant compared to triclosan which exhibited 100% reduction (P value < 0.0001). Against *S. aureus*, chitosan at 92% bacterial reduction had less absolute efficacy than triclosan (100%) but this difference was not statistically significant (P value = 0.29).

4. Conclusions

The results of this study show that treatment of polyester/cotton substrate with the naturally occurring antimicrobial agent, chitosan, imparted antimicrobial activity against both *S. aureus* and *E. coli*. The treatment was very durable to laundering against the Gram positive bacteria but less durable against the Gram negative bacteria. The research thus suggests that chitosan based natural antimicrobial agent has the potential to be a viable alternative to chemically synthesized antimicrobial agents such as triclosan especially in light of the reported adverse impact of widespread use of chemically synthesized antimicrobial agents.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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