The Evaluation of Antibacterial Activity of Fabrics Impregnated with Dimethyltetradecyl (3-(Trimethoxysilyl) Propyl) Ammonium Chloride

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Abstract

The antibacterial materials such as fabrics, cloths are became important to avoid cross infection by pathogenic microorganisms, especially bacteria such as *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*, to control the infestation by microbes, and to arrest metabolism in microbes in order to reduce the formation odour. Textiles for medical and hygienic use have become important areas in the textile industry. Therefore, to reduce/prevent infections, various antibacterial compounds have been used for all types of textiles. The solutions of disinfectant used are generally active *in vitro*, but, it is also necessary to know the effectiveness of disinfecting cloths in conditions of use. In the current study, it was aimed to determine the antibacterial activity of fabrics functionalized with dimethlytetradecyl (3-(trimethoxysilyl) ammonium chloride compound against *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* bacteria, and also after 5, 10 and 20 wash cycles against *Staphylococcus aureus.* The results of the present study showed that the most susceptible bacterium was *S. aureus* in all standard test methods for unwashed fabrics, antibacterial activity was continued by decreasing even after washing cycles.

Key words: Textile fabrics, dimethlytetradecyl (3-(trimethoxysilyl) ammonium chloride, antibacterial activity, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*

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Introduction

Antibacterial fabrics are important not only in medical applications but also in terms of daily life usage. The application of antimicrobial finishes to textiles can prevent bacterial growth on textiles (Jakimiak et al., 2006). Antibacterial textile production has become increasingly prominent for hygienic and medical applications. The antimicrobial agents can be antibiotics, formaldehyde, heavy metal ions (silver, copper) (Gouda, 2006; Seshadri and Bhat, 2005) quaternary ammonium salts with long hydrocarbon chains (Goldsmith et al., 1954; Lashen, 1971; Seshadri and Bhat, 2005), phenol and oxidizing agents such as chlorine (Goldsmith et al., 1954), chloramines, hydrogen peroxide, ozone (Gouda, 2006). In the present study, fabrics functionalized with dimethlytetradecyl (3-(trimethoxysilyl) ammonium chloride (DTAC) which is a quaternary ammonium compound was examined in view of antibacterial activity.

It was stated by manufacturer that DTAC was reported as highly bacteriostatic and is safe to man and the environment (Aft Corporation, 2005). The compound is comprised of membrane-active microbiostatics. It has been known that the action mechanism of such cationic surfactants is electrostatic interaction and physical disruption (Abel et al., 2002; Ramachandran et al., 2004). The positively

charged ammonium cation of the agent is able to bind to negatively charged sites of the cell-wall surface. As a surfactant, agent is capable of disrupting the membrane and permits the release of electrolytes and nucleic materials, leading to cell death.

Usually, antimicrobial properties can be acquired to textile materials by chemically or physically incorporating functional agents onto fibers or fabrics (Gouda, 2006; Jantas and Górna, 2006). The antimicrobial properties of such textile materials can be durable or temporary. Temporary biocidal properties of fabrics are easy to achieve in finishing, but easy to reduce in laundering. Addition to this, it has been known that antibacterial activity of fabrics during washing cycles and the dosage of antibacterial compound may be reduced or increased according to customer's request. However, the antibacterial agents will vanish completely if they are impregnated in materials without covalent bond linkages (Seshadri and Bhat, 2005; Jantas and Górna, 2006).

The purpose of the present study was to determine the antibacterial activity of fabrics functionalized with dimethlytetradecyl (3-(trimethoxysilyl) ammonium chloride compound against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* bacteria. Since it is important to maintain the antibacterial activity of fabrics in conditions of use, the antibacterial activity of unwashed fabrics was also compared with washed fabrics.

MATERIALS AND METHODS

Preparation of fabrics

Textile fabrics (62% cotton-38% polyester) were purchased from a fabric store (Akın Tekstil A.Ş.) in Lüleburgaz, Kırklareli. Washed fabrics were prepared by commercial washing machines at 40°C for 45-50 minutes using detergent, after washing fabrics were tumble dried.

Determination of antibacterial effects of fabrics

In the current study, the antibacterial effect of the fabrics was determined by EN ISO 20645:2004 (agar diffusion plate test) and AATTC Test Method 147-2004 (parallel streak method). In agar diffusion tests *S. aureus* ATCC 6538, *K. pneumoniae* ATTC 4352, *E. coli* ATCC 10229 and in parallel streak tests *S. aureus* ATCC 6538, *K. pneumoniae* ATTC 4352 strains were used. When effective antibacterial activity was determined in parallel streak method, AATCC 100–2004 Test Method was applied for the determination of reduction in bacteria counts quantitatively.

Test organisms

Lyophilized bacteria were inoculated in an adequate quantity in tryptone soya broth (Oxoid). In order to check the purity of cultures, subcultures were prepared from this suspension on tryptone soya agar (TSA) plate (Oxoid). After incubation 24 hours at 37°C, the identity of bacteria was confirmed by microscopic examination and Gram stain.

From the agar plate culture, a first liquid subculture was prepared. Series of three to four liquid-liquid transfers were maintained throughout the experiment.

EN ISO 20645:2004 (Determination of antibacterial activity-agar diffusion plate test)

Antibacterial fabrics are placed on two-layer agar plates. The lower layer consists of culture medium free from bacteria and the upper layer is inoculated with the test bacteria, individually. For the lower layer 10±0.1 ml TSA were poured into steril Petri plates. After sterilization control, for the upper layer, molten TSA (precooled to approximately 45°C) was inoculated with bacterial culture (1-5 x 10^8), vessel was shaked vigorously to distribute bacteria evenly. Test specimens (∅, 25±5 mm) were imprinted onto the inoculated TSA using sterile forceps. Petri plates were incubated for 18-24 h at 37°C. The level of antibacterial activity was assessed by examining the extent of bacterial growth in the contact zone between

the agar and the test specimen. Inhibition zones were calculated using the following formula:

H= (D - d)/2

where:

H is the inhibition zone in mm

D is the total diameter of specimen and inhibition zone in mm

d is the diameter of specimen in mm.

AATTC Test Method 147-2004 (Parallel streak method)

Using a 4 mm inoculating loop, one loopful of the diluted inoculum was transferred to the surface of TSA plates by making five streaks approximately 60 mm in length, spaced 10 mm apart covering the central area of a standard Petri plates without a refilling of loop. Test specimens were cut with a rectangular die (25X50 mm) and were placed to inoculated TSA transversely across the five inoculum streaks. Petri plates were incubated for 18-24 h at 37°C. Incubated plates were examined for interruption of growth along the streaks of inoculum beneath the specimen and for a clean zone of inhibition beyond its edge. The average width of a zone of inhibition along a streak on either side of the test specimen was calculated using the following equation:

W= (T - D)/2

where:

W is width of clear zone of inhibition in mm

T is total diameter test specimen and clear zone in mm

D is diameter of the test specimen in mm.

AATCC 100–2004 Test Method (Assessment of antibacterial finishes on textile materials)

Test specimens were cut in 4.8±0.1 cm diameters using a steel die. 100 µl working culture were inoculated test specimens, individually in sterile Petri plates. After inoculation, specimens were placed screw cap jar contained 100 ml neutralizing agent (3% Tween 80 and 0.3% lecithin in sterile tap water). The toxicity of neutralizing agent against tested organisms was preexamined and no toxicity was determined. Jars were shaked vigorously for one minute, serial dilutions were made. From each of three suitable dilutions, 0.1 ml liquid was drawn and transferred to TSA. The number of survivors was determined after a 48 hour incubation at 37ºC by counting the colonies as CFU/ml using a Colony Counter Device (åCOLyte Super Colony Counter, Synbiosis).

Furthermore, additional jars were prepared to provide information about the bactericidal activity of the treatment over contact period (60 minutes).

Percent reduction of bacteria by the specimen treatments was calculated using following formula:

R= 100 (B – A)/B

where:

R is % reduction

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

B is the number of bacteria recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation (at “0” contact time).

Swatches of the same fabric construction which containing no antibacterial finish were used as negative control in all experiments.

RESULTS

According to EN ISO 20645:2004 standard test method’s evaluation, ≥1-0 mm inhibition zones and no growth under specimen were accepted as effective, whereas 0 mm inhibition zone and slight growth were evaluated as limited effect. In this sense, treated unwashed fabrics had good effect against *S. aureus* (Figure 1), but after 20 washes the antibacterial effect was insufficient. On the other hand, the susceptibility of *E. coli* and *K. pneumoniae* to agent was similar (Table 1).

Table 1: Antibacterial properties of DTAC treated fabric according to EN ISO 20645:2004 test method

Fabrics	*S. aureus* ATCC 6538	*E. coli* ATCC 11229	*K. pneumoniae* ATCC 4352
	Inhibiton zone (mm)		
Treated antibacterial swatches	3.5±0.04 mm[a]	0 mm and slight growth[b]	0 mm and slight growth[b]
After 5 washes	0 mm and no growth[a]	0 mm and heavy growth[c]	0 mm and heavy growth[c]
After 10 washes	0 mm and no growth[a]	0 mm and heavy growth[c]	0 mm and heavy growth[c]
After 20 washes	0 mm and heavy growth[c]	0 mm and heavy growth[c]	0 mm and heavy growth[c]
Untreated swatches (Control)	0 mm and no growth reduction	0 mm and no growth reduction	0 mm and no growth reduction

[a] Good effect, [b]Limited effect, [c]Insufficient effect

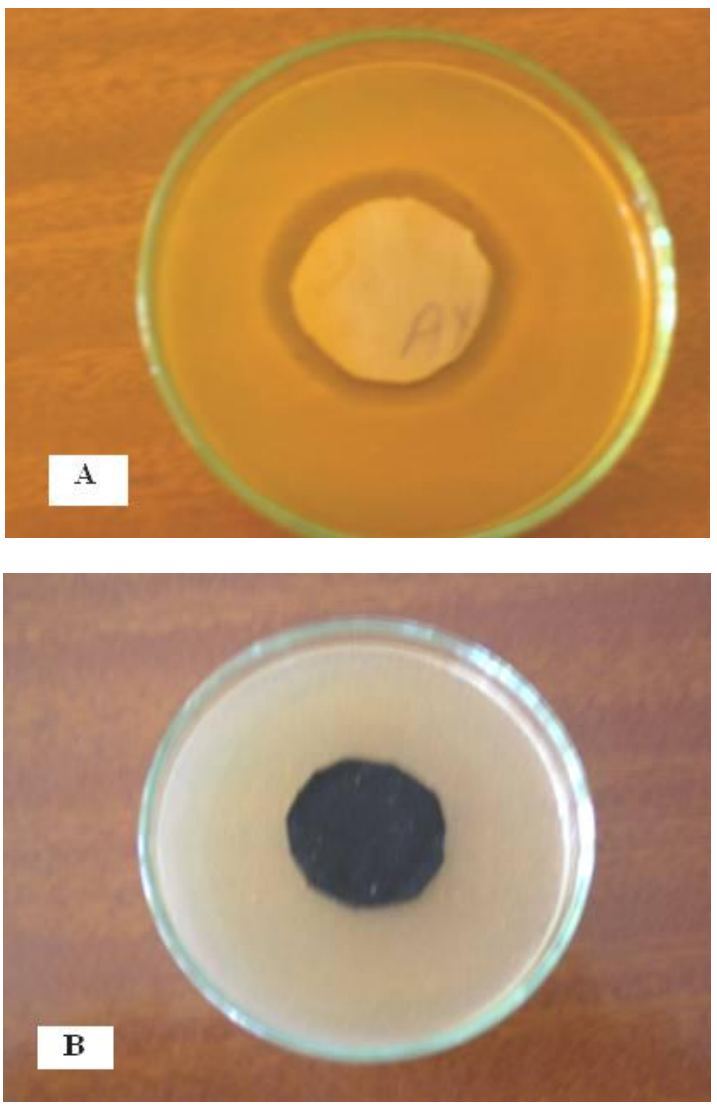

Figure 1. Determination of antibacterial activity by EN ISO 20645:2004 method. A: Antibacterial specimen, B: Control specimen

Table 2. Antibacterial properties of DTAC treated fabric according to AATTC test method 147-2004

	S. aureus ATCC 6538	*K. pneumoniae* ATCC 4352
Fabrics	Inhibiton zone (mm)	
Treated antibacterial swatches	3.5 ±0.0 mm[a]	0 mm and no growth[b]
After 5 washes	0 mm and no growth[b]	0 mm and no growth[b]
After 10 washes	0 mm and slight growth[c]	0 mm and slight growth[c]
After 20 washes	0 mm and heavy growth[d]	0 mm and slight growth[c]
Untreated swatches (Control)	0 mm and no growth reduction	0 mm and no growth reduction

[a] strong antibacterial activity, [b] acceptable antibacterial activity, [c] decreased antibacterial activity ,[d] loss of antibacterial activity

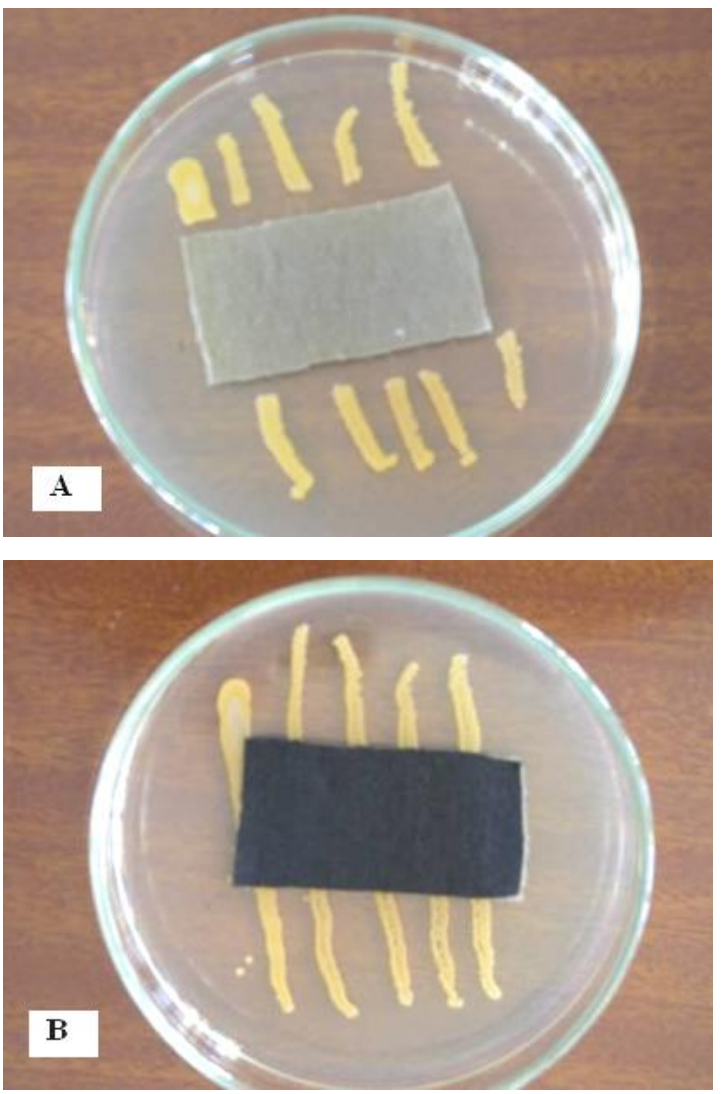

Figure 2: Determination of antibacterial activity by AATTC 147-2004 method. A: Antibacterial specimen, B: Control specimen.

Table 3. Antibacterial properties of DTAC treated fabric according to AATCC 100–2004 test method

Fabrics	*S. aureus* ATCC 6538 Reduction (%) after 60 minutes contact time
Treated antibacterial swatches	98
After 5 washes	20.37
After 10 washes	14.39
After 20 washes	6.20
Untreated swatches (Control)	No growth reduction

Absence of bacterial colonies under the specimen in the contact area is considered as an acceptable antibacterial activity for parallel streak method. After 5 washes, the fabric had showed acceptable antibacterial activity for both *S. aureus* and *K. pneumoniae* (Table 2).

Both of the standard test methods (agar diffusion and parallel streak methods) showed that the most susceptible bacterium was *S. aureus* (Figure 2).

Unwashed antibacterial swatches provided 98% decreases for recoverable *S. aureus* in 60 minutes contact time. It was shown that reduction rates in the viable counts of *S. aureus* in fabrics that were subjected to 5, 10 and 20 washing conditions were 20.37, 14.39 and 6.20%, respectively (Table 3).

DISCUSSION

Molds, yeasts and bacteria can cause discolouration, quality deterioration and formation of odour, by growing on textiles (Birbir, 2003). As much as is the need to protect textile fabrics from microbial attack, antimicrobial treatment for textile materials is also necessary to avoid cross infection by pathogenic microorganisms (Ramachandran et al., 2004). To this end, the antimicrobial agents can be applied to the textile substrates by exhaust, pad-dry-cure, coating, spray and foam techniques (Ramachandran et al., 2004; Jantas and Górna, 2006)

Various test procedures have been used to test the effectiveness of the antibacterial activity, to evaluate antibacterial fabrics, to protect users and textile against action mechanisms of antibacterial compounds (Birbir, 2003). In the current study, EN ISO 20645: 2004 (agar diffusion plate test), AATTC test method 147–2004 (parallel streak method) and AATCC 100–2004 test method were used to examine fabrics functionalized with dimethlytetradecyl (3-(trimethoxysilyl) ammonium chloride (DTAC) in view of antibacterial activity. Agar diffusion test is a preliminary test to detect the diffusive antimicrobial finish. Parallel streak method is suitable for demonstrating bacteriostatic activity by the diffusion of the antibacterial agent. AATCC 100–2004 test method is used for evaluation of fabrics quantitatively, which is showing activity in AATTC test method 147.

In the present study, the most susceptible bacterium was *S. aureus* in all standard test methods for unwashed fabrics. Antibacterial activity of tested fabrics against Gram positive cocci was greater than that against Gram negative rods. This resistance may be attributed to the Gram negative bacteria have very complicated cell walls. On the other hand, Gram positive cocci have a simple cell wall structure in which the cytoplasm membrane has a rigid peptidoglycan layer composed of networks with plenty of pores, which allow foreign molecules to enter the cell without any difficulty (Goldsmith et al., 1954; Lin et al., 2003; Gouda, 2006).

Since DTAC is comprised of membrane-active microbiostatics and shows effect by disrupting, puncturing the cell membrane (Abel et al., 2002; Ramachandran et al., 2004), results of the tests concerning the susceptibility of Gram positive cocci are not astonishing. The susceptibility of *S. aureus* against quaternary ammonium compound is in agreement with the results of Jakimiak et al. (2006).

One of the characteristics of an ideal antibacterial textile is permanent antibacterial activity which is not lost during usage or washing (Birbir, 2003; Lin et al., 2003; Borkow and Gabbay, 2004; Gouda, 2006). Therefore, in the present study, the antibacterial activity of washed fabrics (5, 10 and 20 wash times) was also evaluated. In agar diffusion plate test, fabrics which washed 5 and 10 times were found as having good antibacterial activity against *S. aureus*. On the other hand, in parallel streak tests, acceptable antibacterial activity for 5 wash cycles and decreased antibacterial activity for 10 wash cycles was determined against *S. aureus*.

In quantitative method, reduction of *S. aureus* for unwashed treated antibacterial swatches was 98% and the activity was continued by decreasing even after washing cycles. However, antibacterial fabrics can be recommended, since the fabrics which used in the present study were subjected more washing cycles than those of in terms of daily life usage.

It has been known that *Staphylococcus* has remained viable on surfaces (including clothes) for several months (Engley, 1958) and the main sources for contamination are the patient's skin flora (McNeil et al., 1960). The results described in the present study show that the application of antimicrobial finishes to textiles can prevent bacterial growth on textile.

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