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Original Research Article

Bio-Burden Test: New Approach to Evaluate Efficacy of Corporate Uniform against Human Pathogenic Bacterium.

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Abstract

The study deals with a new approach of evaluate efficacy of corporate uniform against human pathogenic bacterium under different industrial environment. Cotton, polyester /cotton and polyester /viscose fabrics were treated with a silane based quaternary ammonium compound. The antimicrobial efficiency of treated samples measured by BPB stain test shows that the treatment provides good protection against microbes even after 30 washes. The efficacy of treated aprons against human pathogenic bacterium found in different industrial environment were evaluated by bio-burden test also shows low bacterial loads in treated textiles compared to the untreated ones.

Keywords: Antibacterial Efficacy, Bio-burden test, BPB test, Silane based QAC, Uniform fabrics.

1. Introduction

Now a days textile consumer all around the world becoming much more aware of the deleterious effects, that micro-organism may have upon textiles and up on human hygiene. Textiles are one of the main bacteria carrying medium. Textile fibres provide the perfect platform for growth of microorganisms. Even the different environments (hot, humid, and cold) lead to growth of different bacteria. These bacteria/microbes on textiles often result in staining, decolouring of the fabric and leads to bad odour [1-6]. In India, the use of antimicrobial textiles is become prime necessity due to the worm and humid climate. This is the most suitable environment for the growth of microorganisms. Clothing and textile materials are the carriers of these microorganisms such as pathogenic bacteria, odour generating bacteria and mould fungi, also become good media for their growth[7-10].

Microbial infestation poses danger to both living and non living matters. Obnoxious smell form the inner garments such as socks, spread of diseases, staining and degradation of textiles are some of the detrimental effects of bad microbes.

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Though the use of antimicrobials have been known for the decades, it is only in the recent couple of years several attempts have been made on finishing textiles with antimicrobial compounds[11-20]. Antimicrobial finish is a recent innovation in finishes. The consumers are now increasingly aware of the hygienic life style and there is a necessity and expectation for a wide range of textile products finished with antimicrobial properties[21-25]. This finish prevents the growth of bacteria and products finished in it have been proved environment friendly and health protecting, preventing diseases. It also prevents garments from unpleasant odour [26-36].

Antimicrobial efficiency of textiles can be measured by various methods i.e. Bromo phenol blue method, Agar based zone of inhibition test, Bacteria counting test, Soil burial test. The selection of an antibacterial test method is depends upon endues application of the material. If antibacterial efficacy is required, the preferential methods to use are the Cell Suspension tests, as they reflect actual use conditions. The qualitative and quantitative evaluation of antimicrobial treated fabrics can be performed as per

AATCC and ASTM standard. In this study an attempt has been made to evaluate the anti bacterial efficacy of industrial aprons by Bio-burden test.

The experimental plan was based on application of a silane based QAC namely [2-Hydroxyethoxydimethoxysilyl] Octadecyldimethyl Ammonium Chloride (Antimicrobial agent) on polyester/viscose, polyester/cotton blend and 100%cotton fabric by pad–dry-cure method. The antimicrobial activity of treated fabric was evaluated by BPB (Bromo Phenol Blue) test. The antimicrobial efficacy of the treated fabrics was further evaluated after 30 wash according to the standard method. This silane based QAC product was also applied to industrial apron made of polyester/viscose blend fabric by exhaust method. These aprons were exposed in different industrial environment like milk industry, food processing unit, hospital, pathogenic lab, etc. The aprons were collected after 3 to 6 days and finally tested for its antibacterial efficiency using Bio-Burden Test.

2. Materials and Methods

2.1 Materials

2.1.1 Fabrics

Three types of uniform fabrics selected were polyester/viscose (P/V), polyester/cotton (P/C) blend and 100% cotton (C). The detail specifications for fabrics are given in Table 1, supplied by Kiran Threads, Vapi (Gujarat), was used in the present investigation. The fabric was further treated with 5% soap lisapol L, and 2% soda ash at boil for 15 min., to remove the fished, thoroughly washed, neutralized and air-dried before use.

2.1.2 Chemicals

A silane based QAC namely [2-Hydroxyethoxydimethoxysilyl] Octadecyldimethyl Ammonium Chloride (Antimicrobial agent) commercially known as *Zycrobial* was used as antibacterial finishing agent supplied by Zydex Industries limited, Vadodara. Acetic acid (CH_3COOH), R-77 Sodium carbonate (Na_2CO_3) and ECE reference detergent was used without further purification. All chemicals used in this experiment were of analytical grade and used without further purification.

2.2 Methods

2.2.1 Preparation of textile fabrics for treatment

To remove the finish and other hydrophobic impurities from all the three selected fabrics. The fabrics were treated with the bath containing 5 gpl non-ionic detergent (R-77) and 2 gpl sodium carbonate for 30 minutes at 80^0C temperature. The fabrics were then washed thoroughly in running water, neutralized, washed again in running water and finally dried under shade. The pretreatment process was carried out in L.G. Direct Drive washing Machine. The pH of fabrics was checked to neutral before further processing.

2.2.2 Application of antimicrobial agent to textiles by padding technique

The antimicrobial agent was applied to fabric by padding technique. Fabric was immersed in liquor containing 30 gpl antimicrobial agent for 10 minutes subsequently pass through the padding mangle at 2.5 kg/cm^2 pressure using laboratory two bowl padding mangle. Finally the fabric was dried at room temperature.

2.2.3 Application of antimicrobial agent to aprons by exhaust technique

In exhaust application, the uniform garments were treated with 3% and 5% (owf) antimicrobial agent for 20 minutes at room temperature keeping the Liquor ratio 1:10. The 5% (owf) treatment was carried out for hospital and pathology lab aprons and other aprons were treated with 3% (owf) antimicrobial agent. The treatment was performed in washing machine using exhaustion technique of application. Finally, the samples were dried at room temperature under shade.

2.2.4 Washing process

The durability of the antimicrobial treatment was evaluated by BS EN 26330:1994 method using domestic washing process. The specimen was washed in an automatic domestic washing machine by using 1 gpl ECE detergent at 40^0C for 30 minutes and line dried at room temperature according to specified procedure. The process was repeated for 30 times using the same procedure of washing. After 30 wash, the samples were tested for their efficacy by BPB test.

2.2.5 Evaluation of treatment for antibacterial activity of textiles

Antibacterial efficiency of textiles was measured by two methods:

a. Bromo phenol blue method (BPB-Stain Test)

b. Bio-Burden Test

2.2.5.1 Evaluation of antimicrobial activity by BPB methods

Testing of white or light- coloured goods:

- Bromophonol Blue (BPB) solution of 0.025% was prepared in distilled water; few drops of saturated Na_2CO_3 solution per 100 ml BPB solutions was add.
- 10 cc of the solution was taken in beaker and the test specimen was soaked in the solution for 20 mins. Finally the sample was rinsed in distilled water.
- The sample was observed for the blue stain and compare against Bramophenol Blue colour test scale.

2.2.5.2 Evaluation of antibacterial efficiency by Bio-Burden test

Bio-burden is new test method for testing antimicrobial activity of textile. Bio-burden is normally defined as the number of bacteria living on a surface (Textile, Food, etc.) that has not been sterilized. The term is most often used in the context of bio-burden testing, also known as microbial limit testing, which is performed on pharmaceutical products, medical products and membrane filtration for quality control purposes. Products or components used in the pharmaceutical or medical field require control of microbial levels during processing and handling. Bio-burden or microbial limit testing on these products proves that these requirements have been met.

The population of viable microorganisms (bio-burden) in a product or on a product surface is required to monitor a production process, be it for a medicinal product or a medical device. In most cases, with a medicinal product one is examining the product directly, in which case there is a need to make sure there are no antimicrobial properties in the product to affect the results. This is normally done by microorganism recovery experiments. In the case of medical devices there is the possibility that the extraction procedure may not remove all the microorganisms from the device, thereby causing an underestimation of the actual bio-burden present. These are based upon the standard ISO 11737:1[27-28].

Bacteriological studies of efficacy of the antibacterial activity on treated and untreated uniform garments after use were following steps:

- Two sets of treated and untreated aprons (Shown in Table 2) were distributed for use in different environmental settings like
  - Hospital
  - pathology laboratory
  - Milk product factory
  - Food processing unit
  - General Chemical Laboratory.
- After use for 3-6 days by the staff members in the respective environments, the aprons were collected in sterile polyethylene bags and brought to the bacteriology lab.
- A part of the apron material which is likely to be most exposed part (close to the pockets) was cut 2 x 2 cm under sterile conditions.
- Each piece of the cloth was dipped separately into a sterile test tube containing 2 ml nutrient broth solution.
- The tubes were then incubated at 37ºc for 90 minutes.
- With the help of nicrome loop (4 mm diameter), a loop-full of peptone was placed on culture media plates viz., (i) Nutrient agar (ii) Blood agar and (iii) MacConkey's agar.
- The plates were incubated aerobically at 37ºC for over-night or 48 hours.
- The plates were then examined for bacterial growth.
- The isolates were identified to a species level by biochemical tests on an automated instrument (Microscan walk away 41).
- Results were then compared by counting CFU (colony forming units- bacterial load) on treated and untreated cloth.

3. Results and Discussion

3.1 Antimicrobial efficacy of fabrics evaluated by BPB – Stain test

Table 3 shows the antimicrobial efficiency of treatment on fabrics. From the results, it can be seen that the samples treated with silane based QAC exhibit good antimicrobial property compared to untreated samples as per BPB stain scale. Efficiency of the treatment was found decreased by increased in number of washing cycle. The result shown in Table 3 revels that after 30 wash, the depth of blue colour stain become lighter than treated samples without wash. Further, the antimicrobial treated cotton samples shows slightly darker colour even after 30 washes. The treated samples shows darker blue stain compared to their untreated counterpart. Even after 30 washes the treated sample shows darker blue stain compared to the untreated control sample. This result indicates that the efficiency of silane based QAC treated samples retained antimicrobial property even after 30 wash.

3.2 Efficiency of silane based QAC treated aprons by Bio-burden test

The antimicrobial treated and untreated aprons were tested in microbiology lab after exposed to different environment namely, Dairy (Butter and Milk section for 6 days i.e. 3-3 days in each section), Dairy (Paneer and Ice-cream section for 6 days i.e. 3-3 days in each section), Restaurant (for 3 days), Bakery (for 3 days), General environment i.e in chemical lab (for 3 days), Hospital ward (for 6 days) and pathology testing lab (for 6 days). All aprons testing report are shown in Table 4 and Table 5. The results shown in Table 5 are in cfu/cm^2 which is converted in percentage reduction of bacterial compared to the silane based QAC treated aprons. The results for untreated aprons exposed to each environment shown in Table 5.

In **Hospital environment**, bacterial reduction was 100% i.e. no colony was found in silane based QAC treated apron compared to the untreated apron where *Staphylococcus* (non-hemolytic, coagulase negative) bacterium was found. In **pathology lab environment**, bacterial reduction was found 66.67%. The colony count in treated apron was 10,000 cfu/cm^2 of *Bacillus subtilis* bacterium and in untreated it was increased to 30,000 cfu/cm^2 of *Staphylococcus* (non-hemolytic, coagulase negative) and *Bacillus subtilis* bacterium.

In **dairy (butter and milk processing section),** bacterial reduction was 100% i.e. no colony was found in treated apron but in untreated apron *Bacillus subtilis* bacterium was found. In **dairy (paneer and ice-cream processing section)**, bacterial reduction was 80%. The colony count in silane based QAC treated was found 10,000cfu/cm^2 compared to the untreated aprons where it was increased to 50,000 cfu/cm^2 of *Bacillus subtilis* bacterium. In r**estaurant and bakery**, bacterial reduction was 100% i.e. no colony was found in treated apron but in untreated apron growth of *Staphylococcus* (non-hemolytic, coagulase negative) and *Bacillus subtilis* bacterium was observed. In **general environment** i.e. chemical lab, bacterial count in untreated fabric itself is low i.e. only 100 colony. So that silane based QAC treatment was easily resist the growth of these bacteria.

Conclusions

A quaternary amine based antimicrobial agent was applied successfully by economical pad-batch technique on cotton, polyester/cotton and polyester/viscose blend fabric. The treatment was found to improve the antimicrobial efficiency of cotton, p/v and p/c fabric evaluated by BPB stain test. Silane based QAC treated aprons were exposed to different environment and evaluated by bio-burden test. The treated aprons shows low bacterial loads in different industrial environment compared to their untreated counterpart. Generally the bio burden test was utilized for the evaluation of bacterial load in food and pharmaceutical industries but it can also be utilize successfully in the evaluation of antimicrobial efficacy of textiles.

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Table 1 Specification of fabrics.

Specification	Fabrics		
	P/V	**P/C**	**C**
Weave	Plain	Plain	2/1 Twill
Blend (%)	80/20	67/33	100% C
GSM	175.24	119.57	246.77
EPI/ PPI	58/50	100/76	78/53
Count/Denier	416/380	161/155	14.8/11.5
Width (cm)	148.5	92	152
Thickness (mm)	0.38	0.30	0.62

Table 2 Details of the aprons exposed in the different environment

Sr.No.	Treated/Untreated	Description of Environment	Days to Exposed
1	Treated	Dairy-(Butter, Milk)	6
	Untreated	Dairy-(Butter, Milk)	6
2	Treated	Dairy-(Paneer, Ice cream)	6
	Untreated	Dairy-(Paneer, Ice cream)	6
3	Treated	Restaurant	3
	Untreated	Restaurant	3
4	Treated	Bakery	3
	Untreated	Bakery	3
5	Treated	General Env. (Zydex Lab)	3
	Untreated	General Env. (Zydex Lab)	3
6	Treated	Hospital	6
	Untreated	Hospital	6
7	Treated	Pathology Lab	6
	Untreated	Pathology Lab	6

Table 3 BPB Stain test result of silane based QAC treated and untreated fabrics

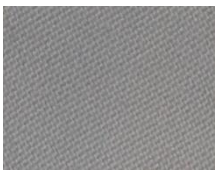
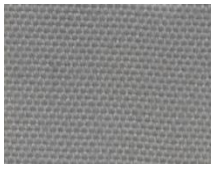
Treatment	P/V	P/C	Cotton
Untreated			
	– –	– –	– –
30 gpl Silane based QAC			
	+ +	**+ +**	**+ + +**
30 wash treated			
	+	**+**	**+**

Table 4 Bio-burden test results for treated and untreated P/V aprons exposed to different environment

Environment	**Untreated p/v uniform result (cfu/cm^2)**	**Treated p/v uniform result (cfu/cm^2)**
Dairy (Butter and Milk)	2×10^4	0
Dairy (Paneer and Ice-cream)	5×10^4	1×10^4
Restaurant	2×10^4	0
Bakery	3.1×10^4	0
General Environment (Chemical Lab)	1×10^2	0
Hospital	10×10^3	0
Pathology Lab	3×10^4	1×10^4

Table 5 Reduction in bacterial growth in treated P/V apron evaluated by Bio-burden test

Name of the Environment	**Bacterial reduction in %**	**Remarks (Antibacterial activity)**
Dairy (Butter and Milk)	100	Excellent
Dairy (Paneer and Ice-cream)	80	Good
Restaurant	100	Excellent
Bakery	100	Excellent
General Environment (Chemical Lab)	100	Excellent
Hospital	100	Excellent
Pathology Lab	66.67	Good

Source of Support: Nil.

Conflict of Interest: None declared

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