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SYNERGISTIC ACTIVITY OF OFLOXACIN AND ORNIDAZOLE ON BIOMEDICAL FABRICS AGAINST NOSOCOMIAL PATHOGENS

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ABSTRACT : Nosocomial infections in the hospitals disseminated from the cotton fabrics of health care professionals and patients leads to severe complications like respiratory, gastrointestinal and urinary tract infections. Since the hospital based textile materials like nylon and polyester has good surface properties, it can harbour large number of microorganisms. Hence in this study, two different antibacterial drugs showing synergistic properties were attached to different fabricsusing tocopherol acetate as a cross-linker with the aim that, treated fabric could act as barriers against transmission of challenge organisms. Inorder to decrease the drug resistant property of the nosocomial pathogens, a fluoroquinolone and a nitroimidazole compounds were mixed at suitable composition based on their synergistic behaviour. Both the compounds were modified to act as reactive dyes and were covalently bonded to the surface of nylon and polyester in order to impart antibacterial properties. The assay used for measuring antibacterial properties was based on the AATCC Test Method-100. The treated fabric was also subjected to multiple washings to determine its durability based on the AATCC Test Method-124. To determine the mode of action of these drugs, DNA of the drug exposed and unexposed challenge organisms were extracted and analysed by agarose gel electrophoresis. The difference in the number of viable bacteria after '0' contact time and 18 hours contact time with treated fabrics were statistically calculated with $P<0.05$ considered significant.

Key words: Reactive dye method, AATCC-100, AATCC-124, beta-cyclodextrin, agarose gel electrophoresis.

INTRODUCTION

Many factors promote infection among hospitalized patients: decreased immunity among patients; the increasing variety of medical procedures and invasive techniques creating potential routes of infection; and the transmission of drug-resistant bacteria among crowded hospital populations, where poor infection control practices may facilitate transmission like contact of wound infection with fabric. Fabric can act as a "harbor" by offering ideal environment for medically significant microorganism like *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Enterobacter*aerogens and *Streptococcus pyogens*. The only effective strategy for reducing such infections and conditions for reservoir of organisms where resistance is stimulated is to reduce the dose of microorganisms throughout the healthcare complex using safe persistent antimicrobial technologies to treat such surfaces and to maintain the highest standards of hygiene (Curtis White & Monticello, 2002).

More recently, an awareness of general sanitation, contact disease transmission, and personal protection have led to the development of antibacterial fibres to protect wearers against the spread of bacteria and diseases rather than to protect the quality and durability of the textile material. Most of these approaches entail the attachment of a biocidal or bacteriostatic agent to the fabric surface. The mechanisms used to attach these agents to the fabric include the layer deposition of silver nanoparticles onto fabric structures (Dubas*et al*., 2006), graft polymerization of N-halamide monomers onto cellulosic substrates (Lui and Sun, 2006), placement of quaternary ammonium salts onto cotton fabrics using a covalently bound adduct (Son *et al*., 2006), covalent attachment of a chloromelamine derivative (Sun *et al*., 2005), and the attachment of chitosan to cotton fabric via cross-linking agents (Eltalawy*et al*., 2005; Ye *et al*., 2006).

This study looked at the feasibility of utilizing two common antibacterial drugs and chemically converting them in order to obtain a reactive dye type molecule, which could be applied to textile fabric materials (nylon, polyester) with the goal of imparting the antibacterial properties of the antibacterial drugs to the fabric. The two compounds used were ofloxacin and ornidazole, possess bacteriostatic properties effective against a wide range of bacteria including *Escherichia coli, Enterobacter*, *Klebsiella*, *Salmonella*, *Shigella*, *Citrobacter* and *Proteus* species (Bharadwaj, *et al*., 2003). Ofloxacin inhibits the activity of the bacterial enzyme DNA gyrase, which is responsible for the negative supercoiling of DNA, an essential conformation for DNA replication in the intact cell (Sato*et al*., 1986). The 5-nitro group of ornidazole undergoes reductive transformation to an active intermediate, which then exerts an inhibitory or lethal effect against DNA. Not only the DNA synthesis inhibited but also causes a loss of the helical structure of DNA with subsequent DNA strand breakage (JurgWust, 1977). Since both drug acts on the similar target site (DNA) of bacteria, it proves the character of synergism. Before applying the compounds in the fabric, the agents were analyzed for their synergistic properties *in vitro.* The synergistic effect can be tested by using the calculative value of MIC of individual agents against the pathogens. Using fractional inhibitory concentration index, the synergistic effect of the two compounds can be identified. These two compounds were reacted with biodegradable tocopherol acetate, and the resultant product was then cross-linked to hospital fabrics using an exhaust methodology. Treated fabrics were then assayed for antibacterial properties based on the AATCC Test Method 100-1999. To check the durability of finished textiles wash-fast test was carried out followed by testing its antibacterial activity using the standard AATCCtest method 100-1999.

MATERIALS AND METHODS

In the present research, testing the synergistic activity of antimicrobial drugs and effect of drugs on bacterial DNA tests were carried out in Microbiology laboratory, CMS College of Science and Commerce, Coimbatore, India, from January 2011 to February 2011. Reactive dye method and Antibacterial activity of treated fabric materials (AATCC 100 method)were carried out in Microbiology laboratory, PSG College of Arts and Science, Coimbatore, India, in March 2011.

Textile materials

The fabric from a commercial producer used for various purposes in the healthcare centre was used as the test fabric. 100% polyester and 100% nylon was selected and sterilized prior experimentation. The fabric was cut into squares (swatches), approximately 5 cm x 5 cm, before being treated. After treatment, the swatches were wrinkled removed and sterilized in prior to the antibacterial assay.

Clinical isolates (90 S agitation method)

Clinical pathogens were isolated from the contaminated hospital used fabrics by the method described by Cody *et al*., 1984. Briefly, Circular swatches with an area of 5 cm^2 werecut from the stained fabric (blood and body tissue fluid) and placed into 100ml of TSB broth in a sterile flask. The flask was sealed and its contents were then shaken for 90 s in a shaker at 580rpm. 10 ml of broth were vacuum filtered through sterile 0.45-µm-pore-size, 47–mm-diameter nitrocellulose filters. Each filter was rinsed twice with 5 ml of sterile PBS and then placed directly on to Trypticase soy agar with 5% sheep blood and other selective media for identifications.

Antimicrobial compounds

Medical grade fluoroquinolone compound (ofloxacin) and nitroimidazole compound (ornidazole) were purchased from Sigma chemical Co. The drugs were checked for their purity based on their specific wavelength using UV-VIS spectrophotometer.

Cross-linker or drug-carrier

Biodegradable cross-linkers or drug-carrier was used to treat the textile materials based on their mode of application. For treating the textile materials, two common cross-linkers were used. Food and medical grade tocopherol acetate (Hi Media) were selected in the study for their biodegradable properties.

Minimal Inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) of each drugs were determined by a standard agar dilution method as described by Qaziasgar and Kermanshahi (2008). Subsequently by doing a checkerboard titration, the combined action these set of drugs on each challenge organism was also studied to assess the synergistic effect of the drugs using fractional inhibitory concentration index (FICI) (Bharadwaj*et al*., 2003).

Checker board titration method (Qaziasgar and Kermanshahi, 2008)

To assess antimicrobial combinations in vitro the checkerboard method was selected. In this technique by using broth dilution method, the concentrations tested for each antimicrobial agent were typically ranged from four or five below the expected MIC to twice the anticipated MIC as in the 45 degree line in Figure-1 (each square represents one plate). 96 well microtitre plate was arranged in such a way that, the wells along the left hand column contains gradient concentrations of first drug (ofloxacin) and the wells along the bottom row contains gradient concentration of other drug (ornidazole). 100 µl of challenge organism was added to all the wells and incubated for 24 hours to observe the growth. The growth pattern in micro dilution wells was detected and interpreted by optical reading, the amount of light transmitted through each well with the ELISA reader system.

Fractional inhibitory concentration index

The FIC index was then calculated by using the following equation by summing the separate FICs for each of the drugs present in that well

Formula to determine synergy

$$FIC_A = \frac{MIC_A \text{ in combination}}{MIC_A} \qquad FIC_B = \frac{MIC_B \text{ in combination}}{MIC_B}$$

Where A and B are the two antimicrobials under investigation

$$FICI = FIC_A + FIC_B$$

Interpretation

Synergy= mean FICI < 0.5

Antagonism= mean FICI > 2.0

Synergy:Synergistic action of a combination of antibiotics is present if the effect of the combination exceeds the additive effects of the individual components.

Antagonism:Antagonism is present if a reduced effect of a combination of antibiotics is observed in comparison with the effect of the most effective individual substance.

Methods of treating synergistic drugs

In the present research a method was described to treat the synergistic drugs with hospital based textile fabrics, polyester and nylon. The method was as described by Chun and Gamble, (2007) for reactive dye method.

Reactive dye method (Chun and Gamble, 2007)

Synthesis of reactive synergistic drugs using tocopherol acetate(TA)

Synthesis of reactive drug-A (ofloxacin) was accomplished by suspending 400 mg ofloxacin in 2 ml deionized water in an ice bath at 5° C. To this suspension, 2 ml tocopherol acetate was added. The suspension was maintained at 5° C during the drop-wise addition of 1.0 N NaOH to dissolve completely.

Synthesis of reactive drug-B (ornidazole) was accomplished by suspending 200 mg ornidazole in 2 ml deionized water in an ice bath at 5°C. To this suspension, 2 ml tocopherol acetate was added. The suspension was maintained at 5°C during the drop-wise addition of 1.0 N NaOH.

Dye-exhaust method to bind reactive antibacterial agents to polyester and nylon fabric

An exhaust dyeing method was used to bind the reactive drug to the polyester fabric. The dye bath was prepared by adding 0.2 ml of Triton-X-100 (Hi media), 2g of sodium sulfate, and 2ml of the any of the reactive drug to 100 ml of deionized water. Three, 5 X 5 cm squares of the fabric were submerged in the dye bath heated to 60 °C. After 30 min of incubation, 1.0 g NaOH that had been dissolved in 10 ml of deionized water was added. The temperature was then raised to 80 °C, and the fabrics heated for another 30 min. The fabric was then rinsed in deionized water and heated for 10 min at 80 °C in deionized water, then rinsed and kept in a convection oven at 105 °C until dried. Similar experimental set up was carried out for nylon fabric with the combinations of synergistic drugs.

Assay for antibacterial properties (AATCC Method 100-1999)

All the treated fabrics and untreated fabrics by both the methods were subjected to antibacterial assay. The assay used for measuring antibacterial properties was based on the AATCC Test Method 100-1999 as described by Rajendran*et al*., 2010. Briefly, 1.0 ml of 12 hours challenge bacterial inoculum was dispersed as droplets over the 3 swatches using a micropipette. The swatches were inoculated in pre-sterilized 250 ml erlenmeyer flasks. After all the samples were inoculated, the flasks were incubated at 37 ± 2 °C for 18 h before being assayed for bacterial population density. The bacterial population density was determined by extracting the bacteria from the fabric by adding 100 ml of distilled water to each flask and shaken using an orbital shaker for 1 min. Then aliquots were serially diluted and pour plated to determine the bacterial density (Chun and Perkins, 1996). The difference in number of viable bacteria was evaluated on the basis of the percentage reduction. Percentage reduction was calculated using the following formula.

$$R = (A-B) / A \; X \; 100$$

Where *R* is percentage reduction, *A* is the number of bacteria in the broth inoculated with treated test fabric sample immediately after inoculation i.e., at zero contact time and *B* is the number of bacteria recovered from the broth inoculated with treated test fabric sample after the desired contact period – 18 hours.

Wash fastness test (AATCC Test Method 147)

Wash fast test was conducted based on the method described by Rajendran*etal*., 2010 to see if the drug would persist through multiple washings. The fabric was washed based on the AATCC Test Method-124 laundering procedure, which used a normal/cotton sturdy cycle, 1.81 kg (4 lb) load, warm water temperature, and AATCC detergent without optical brightener. The treated and control samples were washed 2 and 5 times, respectively.

Statistical analysis of wash fastness

Using chi-square parameter, the hypothesis was selected as antimicrobial agent has good activity against the challenge organisms. The degree of freedom obtained from the calculated value was V=1. The difference in the number of viable bacteria after '0' contact time and 18 hours contact time were statistically calculated with $P<0.05$ considered significant.

Effect of synergistic drugs on DNA

Since ofloxacin and ornidazole, have a common target action on the bacterial DNA, the present study was conducted to explore the possibility of an *in vitro* synergistic effect of the drugs by checking their efficiency to inhibit DNA synthesis. The purified test cultures of *E. coli* and *S. aureus* were treated to each 200µg of two antimicrobial compounds. Both treated and untreated cultures were incubated overnight at 37ºC. Using a standard method of Sambrook and Maniatis (2001), DNA from both the treated and untreated cultures were extracted. Extracted DNA samples were analysed under UV light after running in 0.8% agarose gel electrophoresis column.

RESULTS

Identification of clinical isolates

The pathogens isolated from the contaminated site of fabric by 90 s agitation method were identified and tabulated in Table-1. Different Gram-positive and Gram-negative strains were isolated. Among them one Gram-positive representative bacterium and one Gram-negative representative bacterium were chosen for the present study. *Staphylococcusaureus* and *Escherchiacoli* were used throughout the study based on their significance of nosocomial characterestics.

Table-1: Identification of fabric isolates

Isolates	Cultural characterestics		
	TSB + 5% blood	MacConkey	EMB
Staphylococcus aureus	+	+	_
Escherchia coli	_	+	+

TSB + 5% blood – Partial haemolytic with greenish zones for S. aureus
MacConkey – Lactose fermenting colonies for *S.aureus* and *E.coli*
EMB – Green metallic sheen colonies

Synergistic activity of ofloxacin and ornidazole

All the strains of *Escherichia coli*, and *Staphylococcus aureus* showed complete synergistic effect with ofloxacin and ornidazole combination (Table-2). Also, when mean fractional inhibitory concentrations were calculated for all isolates, only synergy was seen (Table-3).

Table-2: Effect of ofloxacin and ornidazole on fabric isolates

Isolates	Number of isolates				
	S	PS	NE	A	Total
Escherichia coli	2	0	0	0	2
Staphylococcus aureus	2	0	0	0	2

S: Synergy; PS: Partial synergy; NE: No effect; A: Antagonism

Table-3: Mean fractional inhibitory concentration index (FICI)

Isolates	FICI	Interpretation
Escherichia coli	0.5	synergy
Staphylococcus aureus	0.5	synergy

Synergy= mean FICI < 0.5

Reactive dye method (Chun and Gamble, 2007)

Synthesis of reactive synergistic drugs using tocopherol acetate (TA)

The antimicrobial synergistic drugs used for the research were reactively synthesized with specific cross-linker, tocopherol acetate. The combinations of synergistic drugs with tocopherol acetate for binding with nylon and polyester fabrics were mentioned in Table-4.

Table-4: Application pattern of reactive drugs on textile fabric materials

Synergistic drugs	Cross-linkers	Fabric used	Fabric used
Ofloxacin-ornidazole	tocopherol acetate	Nylon	Polyester

Challenge bacteria used – *Staphylococcus aureus* and *Escherichia coli*

Antibacterial activity of treated fabric material

The antibacterial activities of the fabric material (nylon, polyester) covalently bound with synthesized reactive synergistic drug combinations (ofloxacin-ornidazole) and cross-linker (tocopherol acetate) before and after wash (2, 5 times) were assayed by AATCC 100 method. The table-5 and 6 shows the number of colonies (CFU X 10^3/ml) of challenge bacteria after exposed to the treated fabric materials for the period of 18 hours. Figure-2 and 3 shows the number of viable bacteria (*Escherichia coli* and *Staphylococcus aureus*) grown on treated nylon fabric. Figure- 4 and 5 shows the number of viable bacteria (*Escherichia coli* and *Staphylococcus aureus*) grown on treated polyester fabric.

Table-5: Textiles treated with ofloxacin-ornidazole cross-linked to tocopherol acetate(TA)

Fabric sample treated with ofloxacin-ornidazole	No. of colonies (CFU X 10^3/ml)	
	Nylon+ tocopherol acetate(TA)	polyester+ tocopherol acetate(TA)
Treated fabrics exposed with challenge bacteria at 0^{th} time (before wash)	90	98
Treated fabrics exposed with challenge bacteria after 18 hours (before wash)	11	7
Treated fabrics exposed with challenge bacteria at 0^{th} time (2^{nd} wash)	84	81
Treated fabrics exposed with challenge bacteria after 18 hours (2^{nd} wash)	32	26
Treated fabrics exposed with challenge bacteria at 0^{th} time (5^{th} wash)	83	83
Treated fabrics exposed with challenge bacteria after 18 hours (5^{th} wash)	29	30

Challenge bacteria – *Staphylococcus aureus*

Percentage reduction of challenge bacteria

Fabrics (nylon, polyester) covalently bound with synthesized reactive drugs (ofloxacin-ornidazole) and cross-linker (tocopherol acetate) was assayed for antibacterial activity by AATCC 100 method. Colonies of bacteria recovered on the agar plate was counted. The difference in number of viable bacteria was evaluated on the basis of the percentage reduction. Percentage reduction was calculated using the following formula.

$$R\ (\%) = (A-B) / A \times 100$$

Table-6: Textiles treated with ofloxacin-ornidazole cross-linked to tocopherol acetate(TA)

Fabric sample treated with ofloxacin-ornidazole	No. of colonies (CFU X 10^3/ml)	
	Nylon+ tocopherol acetate(TA)	polyester+ tocopherol acetate(TA)
Treated fabrics exposed with challenge bacteria at 0^{th} time (before wash)	90	92
Treated fabrics exposed with challenge bacteria after 18 hours (before wash)	9	10
Treated fabrics exposed with challenge bacteria at 0^{th} time (2^{nd} wash)	83	88
Treated fabrics exposed with challenge bacteria after 18 hours (2^{nd} wash)	30	29
Treated fabrics exposed with challenge bacteria at 0^{th} time (5^{th} wash)	82	81
Treated fabrics exposed with challenge bacteria after 18 hours (5^{th} wash)	30	29

Challenge bacteria – *Escherichia coli*

Using chi-square parameter, the hypothesis was selected as antimicrobial agent has good activity against the challenge organisms. The degree of freedom obtained from the calculated value was V=1. For all the data, the table value is greater than the calculated value. Hence, the difference in the number of viable bacteria after '0' contact time and 18 hours contact time were statistically calculated with $P<0.05$ considered significant.Effect of initial dose [0 contact time] of *Staphylococcus epidermidis* or *Escherichia coli* for reactive drugs (ofloxacin-ornidazole) on treated nylon fabricswhen compared with final dose [18 hours contact time] for reactive drugs (ofloxacin-ornidazole) on treated nylon fabrics, showed that the numbers of viable bacteria in drug-treated fabric [18 hours contact time] ($P<0.05$) were less than the number of viable bacteria at 0 contact time of treated fabrics ($P>0.05$). Similar statistical result was obtained for the drug treated polyester fabric materials.

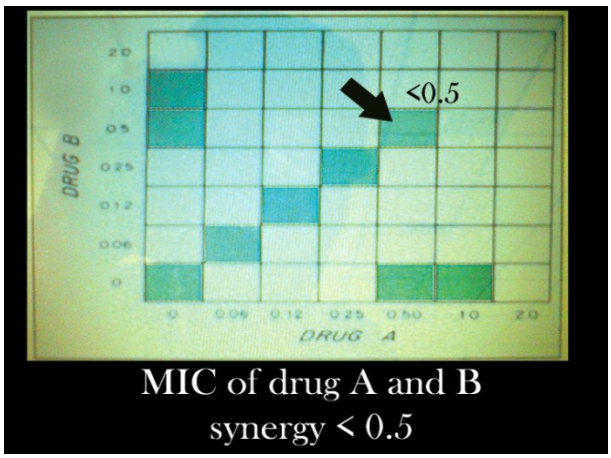

Fig-1: Checker board titration method to evaluate the synergism between two drugs

Diagramatically illustrated the synergism between two drugs: ofloxacin-ornidazole
(Figure extracted from Qaziasgar L and Kermanshahi R.K. 2008. Effect of anti-microbial fiber and its interaction with penicillin G on opportunistic skin micro flora.Iranian journal of basic medical sciences. 11(1): 41-48)

Where *R* is percentage reduction, *A* is the number of bacteria in the broth inoculated with treated test fabric sample immediately after inoculation i.e., at zero contact time and *B* is the number of bacteria recovered from the broth inoculated with treated test fabric sample after the desired contact period – 18 hours

Fig-2: Antibacterial activity of treated fabric material – nylon

Number of colonies of *Escherichia coli* after 18 hours incubation with treated fabric-nylon
5th wash sample plate showing more number of CFU/ml than before wash plate for the challenge organism

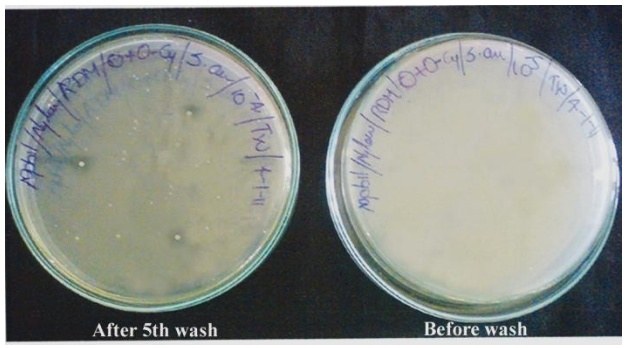

Fig-3: Antibacterial activity of treated fabric material – nylon

Number of colonies of *Staphylococcus aureus* after 18 hours incubation with treated fabric-nylon
5th wash sample plate showing more number of CFU/ml than before wash plate for the challenge organism

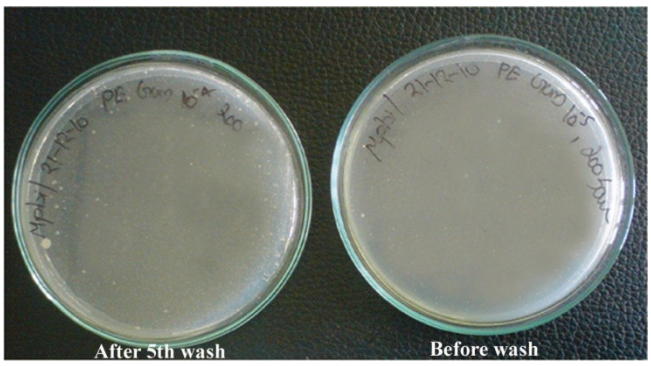

Fig-4: Antibacterial activity of treated fabric material – polyester

Number of colonies of *Escherichia coli* after 18 hours incubation with treated fabric-polyester
5th wash sample plate showing more number of CFU/ml than before wash plate for the challenge organism

Table-7 shows the percentage reduction of the challenge bacteria against the synergistic drugs ofloxacin-ornidazole for both nylon and polyester fabric materials.

Table-7: Percentage reduction of challenge organisms against textile treated with synergistic reactive drugs (Before and after wash)

Samples	Reduction of bacteria (%)					
	S. aureus			*E. coli*		
	before wash	2nd wash	5th wash	before wash	2nd wash	5th wash
Treated nylon exposed with challenge bacteria at 0th time	0	0	0	0	0	0
Treated nylon exposed with challenge bacteria after 18 hours	87	65	61	90	63.8	63.4
Treated polyester exposed with challenge bacteria at 0th time	0	0	0	0	0	0
Treated polyester exposed with challenge bacteria after 18 hours	92.85	67.9	63.8	89.1	67	64.1

From the data (Table-5 and 6) the percentage reduction of challenge organisms against the treated fabrics were calculated and reported in Table-7

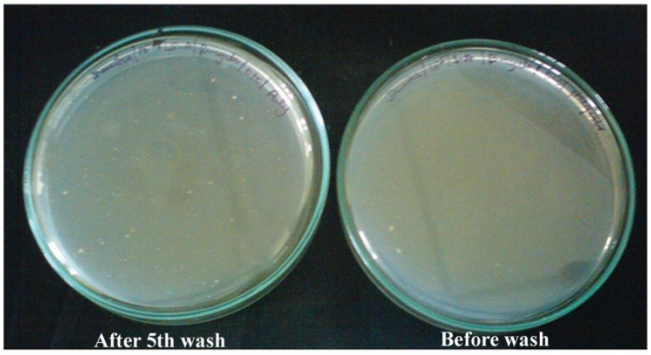

Fig-5: Antibacterial activity of treated fabric material – polyester

Number of colonies of *Staphylococcus aureus* after 18 hours incubation with treated fabric-polyester
5th wash sample plate showing more number of CFU/ml than before wash plate for the challenge organism

Statistical analysis

Using chi-square parameter, the hypothesis was selected as antimicrobial agent has good activity against the challenge organisms. The degree of freedom obtained from the calculated value was V=1. For all the data, the table value is greater than the calculated value. Hence, the difference in the number of viable bacteria after '0' contact time and 18 hours contact time were statistically calculated with $P<0.05$ considered significant.

Effect of synergistic drugs on bacterial DNA

The exposure of bacterial pathogens, *S. aureus* and *E. coli* to ofloxacin-ornidazole was done to understand the interfering activity on the DNA synthesis of challenge organisms. The DNA extracted from the synergistic drug treated and untreated samples were subjected to agarose gel electrophoresis technique. In figure-6 and 7, the difference in the appearance of DNA bands under UV light indicates the difference of drug treated cells from drug untreated cells. The formed DNA band width, the intensity of illumination and UV absorption were more for the ethidium bromide stained DNA bands extracted from untreated than treated. Hence the result revealed the interference rendered by synergistic drugs on DNA synthesis mechanism of the pathogens at time of exposure.

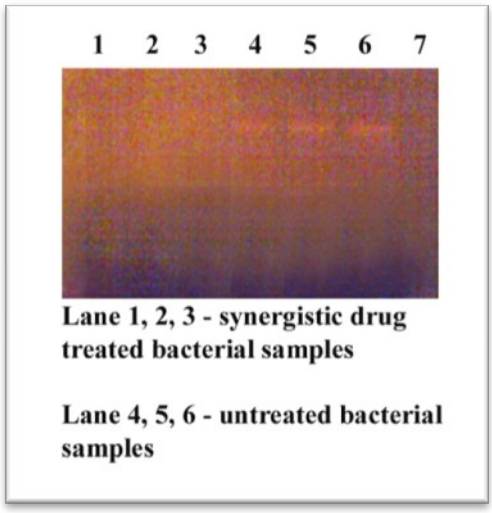

Fig-6: Effect of ofloxacin and ornidazole on DNA of *Escherichia coli*

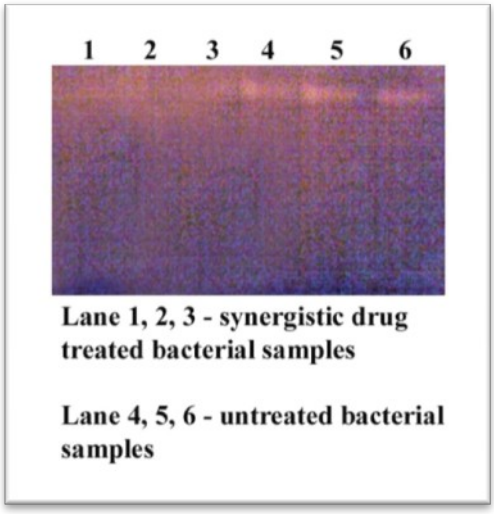

Fig-7: Effect of ofloxacin and ornidazole onDNA of *Staphylococcus aureus*

DISCUSSION

Contaminated hospital used fabrics samples were studied for the recovery of clinical isolates according to 90s agitation method described by Cody *et al*., 1984. The swathes thought of contaminated with hospital pathogen were cut from the cuff regions of long-sleeved fabric and the abdominal region of short-sleeved uniform clothes, bed clothes and patients dress. Using a modified 90-s agitation method, the pathogens from the contaminated swatches were isolated in PBS-TSB broth and identified as *Staphylococcus aureus,* and *Escherichia coli*.

Based on the mode of action of drugs on bacterial components, different combinations of synergistic drugs were selected for the study. According to Bharadwaj*et al*., (2003) and Michael boeckh*et al*., (1990) combination of a quinolone drug with a nitroimidazole drug enhance the antimicrobial properties against both aerobic and anaerobic bacterial infections.
Biodegradable cross-linker (tocopherol acetate(TA)) which was of food grade was selected to treat the fabric materials. According to Jalil and Nixon, 1990, DL-lactic acid and its co-polymers have been known to be biodegradable and histocompatible for the past 20 years.

Their physico-chemical and biological properties have been found suitable, in many instances, for sustaining drug release *in vivo* for days or months. Cyclodextrins (CDs) have been found as potential candidates because of their ability to alter physical, chemical and biological properties of guest molecules through the formation of inclusion complexes (Babootaet *al*., 2003).
Using the specific crosslinker (tocopherol acetate) the antimicrobial drugs were made reactive. The mixture was made at the required concentrations and finally mixed with 1.0N NaOH solutions to make a reactive drug so that the contents can be imparted directly or covalently with the test fabric samples. Similar work was carried out by Chun and Gamble (2007) using cyanuric chloride as cross-linker with trimethoprim-sulfamethoxazole, a synergistic drug combination.
Initial testing determined whether reactive antibiotics would covalently bond to the cotton fabric and impart antibiotic properties to the nylon and polyester fabric materials. A pilot test was done with nylon and polyester treated ofloxacin-ornidazole and norfloxacin-metronidazole against the challenge organisms, *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial activities for all the treated fabrics were determined based on AATCC-100 method as described by Rajendran*et al*., 2010 (before and after wash – AATCC-147 method). The reduction percentage of *S. aureus*, *E. coli* in the nylon fabric treated with reactive ofloxacin-ornidazole (before washing) was 87%, and 90%. The reduction percentage of *S. aureus*, *E. coli* in the polyester fabric treated with reactive ofloxacin-ornidazole (before washing) was 92.8%, and 89.1%. Similar result was noted in the article published by Chun and Gamble (2007). In their experiment, fabric was treated with trimethoprim, sulfamethoxazole, and a 1:1 mixture of trimethoprim and sulfamethoxazole each at half the strength. The observations from three separate antibacterial assays were combined for analysis. The results indicate that both trimethoprim and sulfamethoxazole individually or together depressed the bacterial density of *K. pneumonia* and *S. aureus* significantly after 24-hr incubation. Sulfamethoxazole was less effective than trimethoprim alone or when both trimethoprim and sulfamethoxazole were attached to the fabric.
The large swatches of treated and untreated cotton fabric were washed 2 and 5 times (based on the AATCC-147 method described by Rajendran*et* al., 2010) to determine if the antibiotic binding to the fabric would be durable through normal washing. After washing (5^{th} wash), these large swatches were cut into smaller swatches, sterilized, and then assayed for antibacterial properties. The reduction percentage of *S. aureus*, *E. coli* in the nylon fabric treated with reactive ofloxacin-ornidazole (after 5th wash) was 61% and 63.4%. The reduction percentage of *S. aureus*, *E. coli* in the polyester fabric treated with reactive ofloxacin-ornidazole (after 5th wash) was 63.8% and 64.1%

Using chi-square parameter, the hypothesis was selected as antimicrobial agent has good activity against the challenge organisms. The degree of freedom obtained from the calculated value was V=1. For all the data, the table value is greater than the calculated value. Hence, the difference in the number of viable bacteria after '0' contact time and 18 hours contact time were statistically calculated with $P<0.05$ considered significant. In our study, since the table value is greater than the calculated value, the assigned hypothesis was accepted.

Effect of initial dose [0 contact time] of *Staphylococcus epidermidis* or *Escherichia coli* for reactive drugs (ofloxacin-ornidazole) on treated nylon fabricswhen compared with final dose [18 hours contact time] for reactive drugs (ofloxacin-ornidazole) on treated nylon fabrics, showed that the numbers of viable bacteria in drug-treated fabric [18 hours contact time] ($P<0.05$) were less than the number of viable bacteria at 0 contact time of treated fabrics ($P>0.05$). Similar statistical result was obtained for the drug treated polyester fabric materials.

Similar wash-fastness method was described by Sathianarayanan*et al*., (2010). In their work an ecofriendly natural antibacterial finish has been prepared from the plant extracts for textile application. Herbal extracts from *Ocimum sanctum* (tulsi leaf) and rind of *Punicagranatum* (pomegranate) have been applied to cotton fabric by the method of direct application, micro-encapsulation, resin cross-linking and their combinations. All the treatments show good antibacterial properties for the fabrics. Except the method of direct application, all other treatments show good washing durability up to 15 washes.

In another study conducted by Chun and Gamble, (2007) using pure different synthetic reactive chemicals, trimethoprim and sulfamethoxazole, both unwashed and washed treated fabrics had significantly lower bacterial density than the untreated fabric and the averages were not significantly different among the three treated fabric. This result shows that the treated cotton fabric displayed antibacterial properties that persisted through 10 laundering.

PraneeRattanawaleedirijin*et al*., (2008) reported that antibacterial efficacy of nanosilver finished fabrics on staphylococcus aureus. Bacteriological tests were performed against*S.aureus* as model for gram positive bacteria. The result indicate that the percentage reduction of bacteria in treated fabric was less than 99.9%.Also, the *S. aureus* was completely attracted on the silver finished textile even after being exposed to 20 consecutive hand laundering condition.

Our present study findings agree well with the experimental data reported by Chun and Gamble (2007), Sathianarayanan*et al*., (2010) and PraneeRattanawaleedirijin*et al*., (2008) suggesting that the two synergistic drug combinations as reactive drugs can covalently bind to all fabric materials and inhibits the growth of both Gram-positive and Gram-negative bacterial organisms before wash and after wash (5 repeated laundry washings). With controlled parameters the efficiency of the drugs in inhibiting the organisms can be increased even after 10 consecutive washings.

The formed DNA band width, the intensity of illumination and UV absorption were more for the ethidium bromide stained DNA of the untreated cultures than the antibiotic treated. The test result reveals the inhibition of DNA synthesis was mainly induced by the synergistic effect of both ofloxacin and ornidazole. Ofloxacin inhibits the activity of one of the A-subunits of the bacterial enzyme DNA gyrase, which is responsible for the negative super coiling of DNA, an essential conformation for DNA replication in the intact cell (Dollery, C., 1999). The 5-nitro group of ornidazole undergoes reductive transformation to an active intermediate, which then exerts an inhibitory or lethal effect against DNA. Not only is DNA synthesis inhibited but the reduced metabolite also causes a loss of the helical structure of DNA with subsequent DNA strand breakage (Kucers*et al*., 1997).

Further research must be carried out comparing different concentrations of the antibiotics, both alone and together, in influencing bacterial densities, and the potential influence of competition between dyes for the same hydroxyl groups on textile fabrics. Since ofloxacin, ornidazole were both easily prepared to act as reactive dyes, most of other commonly known antimicrobial compounds having the similar reactive sites may be used in a similar manner, and future research should be expanded to include testing a wide spectrum of antimicrobial compounds.

Conclusion

This creates an opportunity to design or tailor antimicrobial fabric using the reactive dye method to attach antibacterial compounds to polyester and nylon. For example, one area where this approach may prove to be of value would be to attach scarce antibacterial drugs to dressings to act as barriers to specific drug resistant bacteria to help prevent or reduce infection and its spread. In summary, ofloxacin, ornidazole could be prepared as reactive dyes that can covalently bind to textile fabric materials. The treated fabric displayed antibacterial properties that persisted through 5 consecutive launderings. The ease of application may extend to the use of other antimicrobial drugs to provide value to textile fabrics where tailored antibacterial fabric is desired.

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