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Development of advanced personal protective equipment fabrics for protection against slashes and pathogenic bacteria: Part 2: Development of antimicrobial hygiene garments and their characterization.

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Development of Advanced Personal Protective Equipment Garments for Protection against Slashes and Pathogenic Bacteria
Part 2:Development of Antimicrobial Hygiene Garments and Their Characterisation

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ABSTRACT:

Knife is the most commonly used single weapon in the UK and studies reveal that majority of the knife inflicted wounds were slash type that could be disfiguring or life threatening. Currently available stab resistant armours do not protect the arms, neck and face as they are rigid to be worn comfortably for everyday use. The main objectives of this research programme are; a) to develop and characterise a novel cut resistant and slash proof material that is lightweight, comfortable and efficient; and b) to integrate barrier properties in such garments which would incorporate suitable antimicrobial and other suitable chemicals to provide protection against a range of micro organisms. The design and development of novel slash proof materials for the police, armed forces, children and the public, that is lightweight, comfortable and efficient was discussed in Part 1 of this series.

This part discusses the application and study of antimicrobial properties on this novel two-layered weft knitted slash resistant fabric that has only 13.6% of its fibres effectively available for incorporation of antibacterial agents. The anti-bacterial formulation was applied at different concentrations onto the fabric using pad-dry-cure method. Antimicrobial properties of the treated fabrics were evaluated using modified AATCC Test method 147-1998 against common pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli*. It was found that the formulation with 10% concentration showed optimum antimicrobial property with good washing fastness. The durability of the antimicrobial agents were evaluated, both with and without cross-linking agents, for up to 10 washes and better washing fastness was achieved with a cross-linking agent on the face knitted with the composite WF 528 yarn.

KEYWORDS: antimicrobial, microorganisms, personal protective equipment, PPE, garments, test methods, standards.

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INTRODUCTION

The law enforcement and medical personnel require high level of protection when dealing with physical threats. With knives being used more commonly now-a-days, the general public require high level protection from crimes. The first part of this two-part series of papers presented a comprehensive review of the need for protection against slash and stab, the aims of the research programme and the design, development and characterisation of novel slash proof materials for the police, armed forces, children and the public. This paper discusses the application, testing and evaluation of antimicrobial agents to the novel slash proof materials described in Part-I.

It is well established that microorganisms create and aggravate problems by transmitting diseases and infections through clothing, bedding, etc. They exist in large quantities on textile materials and aid in transmitting diseases and infections[1]. Microorganisms cause damage to fibres under normal usage and storage conditions thereby reducing the wear life of the materials[2]. The moisture transport characteristics of fabrics made wholly of synthetic fibres tend to cause a greater degree of 'perspiration wetness'[3] which causes discomfort to the wearer and they retain more odour causing bacteria than natural fibres, especially polyamide fibres[4].

One of the main objectives of the research programme is to incorporate suitable antimicrobial agents into novel materials to provide protection against a range of pathogenic bacteria and to fully characterise the barrier properties of the treated materials. The application of antibacterial agents into the novel slash resistant fabric itself presents a challenge since the fabric has only 13.6% of its fibres by weight (polyamide) effectively available for incorporation of antibacterial agents. The remaining 86.4% of the fabric is made of para-aramid fibres, ultra high molecular weight polyethylene and glass fibres which pose problems imbuing antimicrobial agents into fibre matrix. The slash resistant fabric is designed as a two layered structure, one face formed entirely of Kevlar® aramid fibres and the other face with the engineered composite yarn that is double covered using a polyamide continuous-filament yarn.

A lot of attention has been given to the development of antimicrobial textiles for health-care workers as they are highly exposed to numerous biological hazards in the work place[5,6,7] and numerous papers have already discussed the applications and advancements in antibacterial finishes on textile products[8,9,10,11]. The fibres that are available for incorporating antibacterial agents are made up of polyamide (Nylon6,6) and considerable research has been carried out to successfully incorporate antibacterial agents into the textile structures[12,13]. Different methods of evaluation of antimicrobial finish have been discussed elsewhere[3,14,15,16] and this part, therefore, focuses on the up-to-date results from the study of application of antimicrobial properties on this novel two-layered weft knitted slash resistant fabric that has only 13.6% by weight of fibres available for incorporation of antimicrobial agents.

EXPERIMENTAL WORK

Preparation of Antibacterial Finish

A synergistic system of antimicrobial chemical formulation has been prepared and optimised for maximum antimicrobial activity. All the four chemicals were combined in various proportions in water to form the antimicrobial solution. They were mechanically stirred for 30 minutes to help with even dispersion in water. Formulations with two different proportions of the chemicals were initially evaluated using the Zone of Inhibition test. Since both variations of the proportions showed similar results on both Gram positive and Gram negative bacteria, only one of the combinations of chemicals were used for further studies. The details of the chemicals and the proportions used have been omitted intentionally in order to protect the Intellectual Property Rights (IPR) associated with this innovation.

Preparation of Bacterial Cultures

The antimicrobial properties of the novel slash resistant garments were evaluated using Gram-positive *Staphylococcus aureus* (ATCC 29213) and Gram-negative *Escherichia coli* (ATCC 35218) bacteria. A suspension of each organism was prepared from frozen colonies in a nutrient broth containing 5g/1000ml of Peptone and 3g/1000ml of beef extract. The broth was dispensed in 9.0 ml amounts in conventional bacteriological culture bottles and sterilised for 15 minutes at 120^0C before dispensing the organisms. Further dilutions of the organisms were made to get dilutions of up to 10^{-6}.

Preparation of Test Specimens

The formulation was imbued into the fabric using pad-dry-cure method. The fabric was padded with the antibacterial solution, drip dried and dried at 80^0C for 25 minutes and then cured at 100^0C for 5 minutes. Due to heavy knitted construction of the novel slash resistant garment, it was difficult to cut the fabrics in circles to test the zone of inhibition. Therefore, the test specimens were cut in 2 cm squares using an electric scissor.

Test Procedure

15 ± 2 ml of sterilised nutrient agar was poured into a standard 90mm flat bottomed Petri dish and allowed to gel firmly at room temperature. Contrary to using 4 mm inoculating loop to make 5 parallel steaks on the Petri dish[17], as specified by AATCC Test method 147-1998, 0.5 ml inoculum was spread to cover the full 90mm area of the Petri dish using a disposable spreader. The test specimen was placed at the centre of the Petri dish and pressed gently to make intimate contact with the agar surface.

Due to the two layered bulky structure of the test specimen and to make sure that the fabric is in contact with the inoculum, tests were carried out twice by placing each face of the specimen in contact with the bacterial inoculum. The agar plates were then incubated at 37 ± 2^0C for 24 hours.

Evaluation of Zone of Inhibition

Generally, for a zone of inhibition test the fabric is required to be cut as a circle so as to enable the incorporated chemicals to be leached out evenly in all the directions to arrest or kill the bacteria in the agar medium. Due to the extremely high cut resistance of the developed fabric and the structure of the fabric, it was impossible to cut the fabric as a circle. Therefore the fabric was cut in 2 cm squares and the zone of inhibition was measured in all the four sides and an average was recorded. The size of the zone of inhibition is then compared to that of an untreated control specimen.

Durability Testing

To test the durability of the antimicrobial finish, treated specimens were examined for antimicrobial efficacy after 1, 5 and 10 launderings. The procedure used for laundering is described in the AATCC Test Method 61[18]. AATCC standard reference detergent WOB (without optical brightener) was used[19].

RESULTS AND DISCUSSION

Pick-up of Chemical Formulation

The antibacterial formulation was applied at concentrations of 5%, 10%, 15%, 20% and 25%.

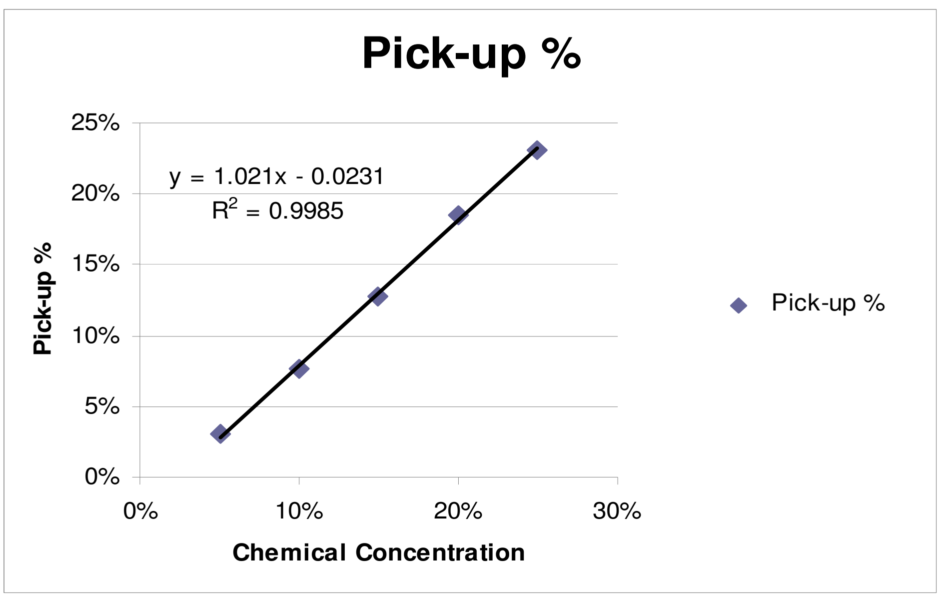


Figure 1: Take-up percentage of formulation at different concentrations

The pick-up percentage of the fabrics at different concentrations was studied to analyse the efficiency of fabric to take up the chemicals and ten specimens were tested for each concentration. It can be seen from Figure 1 that the pick-up percentage varied with the concentration of the recipe and it is linear. Table 1 shows the values of pick-up % for different recipe concentration at 95% confidence limits.

The relationship between the pick-up percentage for the novel slash resistant material and the recipe concentration is calculated by using the linear regression equation-1 with a correlation coefficient (R^2) value of 0.9985.

$$y = 0.0511x - 0.0231 \qquad \text{----- (1)}$$

Table 1: Take-up Percentage of the Formulation at Different Concentrations.

	Recipe Concentration				
	5%	**10%**	**15%**	**20%**	**25%**
Pick-up %	3.01 ± 0.01	7.62 ± 0.01	11.53 ± 0.01	18.55 ± 0.04	23.08 ± 0.07

Antimicrobial Activity against Gram-positive Bacteria

Different dilutions of *Staphylococcus aureus* at 10^{-4}, 10^{-5} and 10^{-6} were studied to test the antimicrobial activity of the slash resistant fabric at of 5%, 10%, 15%, 20% and 25%. Since the fabric specimens could not be fully immersed in the agar solution, the test was conducted separately for both faces of the fabric. Two specimens were tested for each face, giving a total of four specimens and 16 zone of inhibition points. An average of all the 16 points was recorded and the values are shown in Table 2.

The logarithmic trend lines in Figure 2 illustrate that optimum antimicrobial activity is reached at 10% of recipe concentration. It should be mentioned that the rate of antimicrobial activity increases with the increase in concentration of recipe up to 10% and thereafter the increase is not significant. The coefficient of correlation (R^2) value is least for log concentration of 10^{-5} at 0.8083 and is more consistent for lower bacterial concentration of 10^{-6} at 0.9556. It should be noted that these results can not be construed to conclude that the consistency will increase with decrease in bacterial concentration as only three dilutions have been studied.

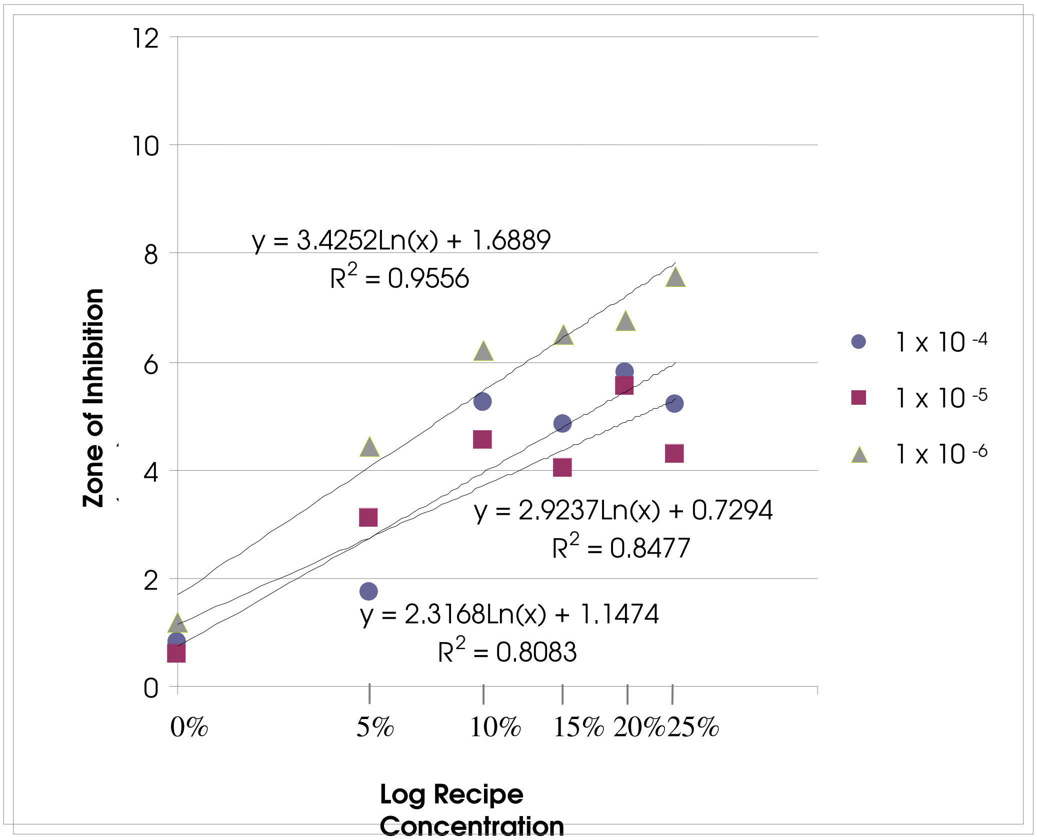


Figure 2: Zone of inhibition using *Staphylococcus aureus*

The antimicrobial effect of the slash resistant fabric on different dilutions of bacteria can be predicted using the following equations:

for 10^{-4} dilution:

$$y = 2.9237 \ln(x) + 0.7294 \qquad \text{------(2)}$$
$$R^2 = 0.8477$$

for 10^{-5} dilution:

$$y = 2.3168 \ln(x) + 1.1474 \qquad \text{------(3)}$$
$$R^2 = 0.8083$$

for 10^{-6} dilution:

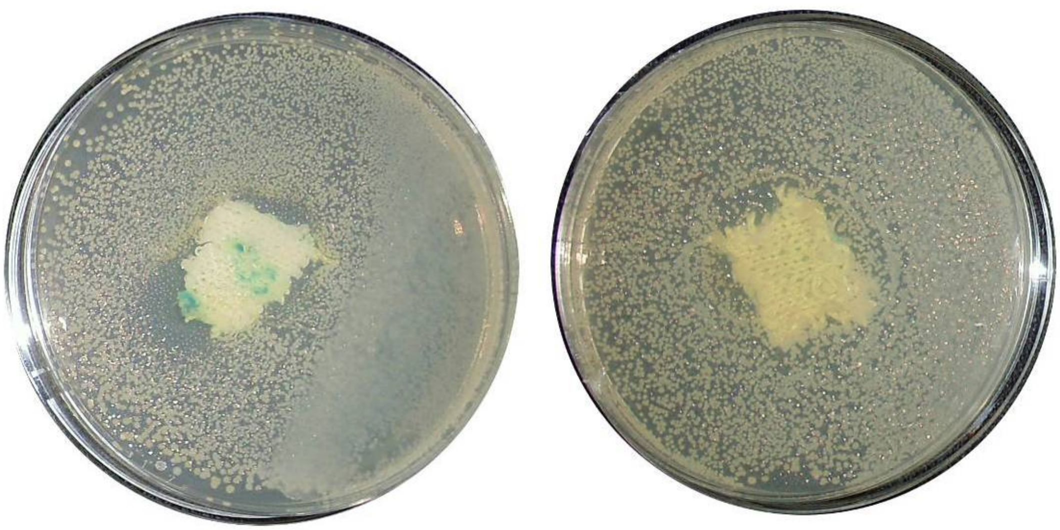
$$y = 3.4252 \ln(x) + 1.6889 \qquad \text{------(4)}$$
$$R^2 = 0.9556$$

where, y = the zone of inhibition in mm; x = antimicrobial chemical concentration; and R^2 = correlation coefficient.

Table 2: Zone of Inhibition using *Staphylococcus aureus*.

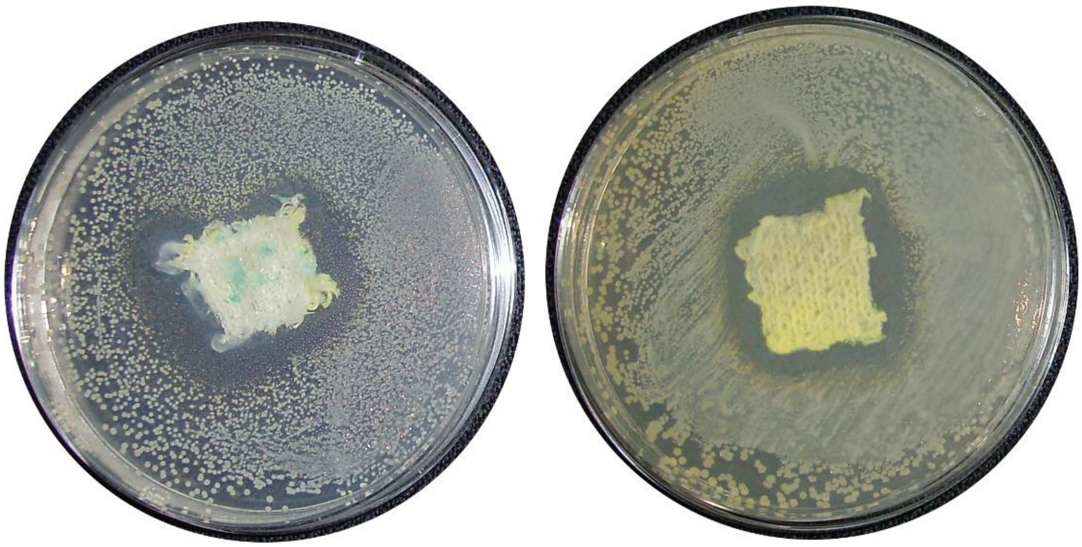
S.Aureus Dilution	Zone of Inhibition (mm)					
	Control	5%	10%	15%	20%	25%
1×10^{-4}	0.8	1.8	5.2	4.8	5.8	5.2
1×10^{-5}	0.6	3.1	4.6	4.0	5.6	4.3
1×10^{-6}	1.2	4.4	6.2	6.5	6.8	7.6

It can be observed from Table 2 that the optimum antibacterial activity can be obtained at 10% concentration with zone of inhibition of 5.2mm, 4.6mm and 6.2mm at various bacterial dilutions. Figure 3 shows the antibacterial activity of the control untreated fabric against *Staphylococcus aureus* at 10^{-4} dilution and Figure 4 shows the antibacterial activity of the fabric treated with 10% concentration of the chemical formulation. The zone of inhibition obtained at 15% concentration (see Table 2) is slightly lower than that of 10% concentration and increases marginally at concentrations of 20% and 25%. Higher concentrations of the formulation could not be tested as the chemicals reach the saturation point at just about 25% concentration.



White face of the fabric   Yellow face of the fabric

Figure 3: Zone of inhibition of control untreated fabric against *Staphylococcus aureus*



White face of the fabric Yellow face of the fabric

Figure 4: Zone of inhibition of treated fabric (10% recipe) against *Staphylococcus aureus*

Antimicrobial Activity against Gram-negative Bacteria

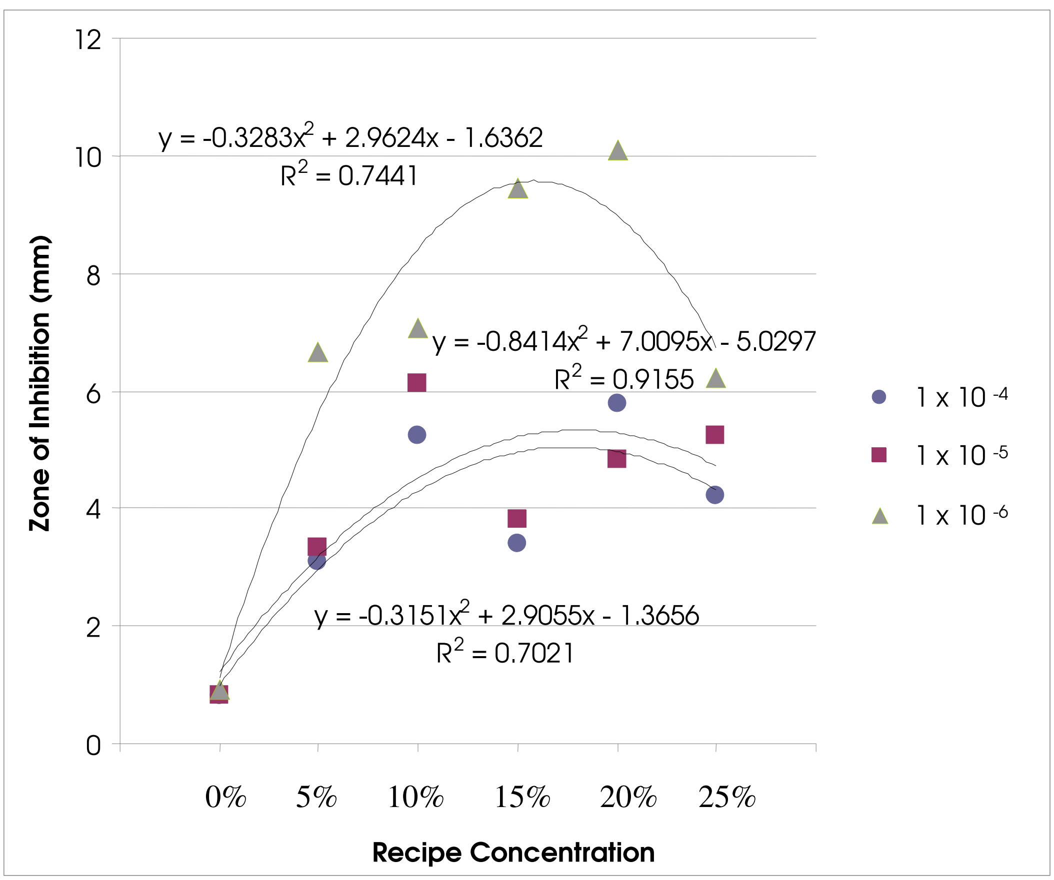


Figure 5: Zone of inhibition using *Escherichia coli*

Figure 5 shows a plot between different bacterial concentrations of *Escherichia coli* and the extent of antimicrobial activity at different concentrations of the synergistic chemical formulation applied on the novel slash resistant fabric. Even though the data does not conform to the relationship between the measured zone of inhibition at different concentrations of chemicals and the log of different concentrations of bacteria, the values shown in Table 3 prove that there is significant antimicrobial activity against Gram-positive bacteria. Figure 6 shows the antibacterial activity of the control untreated fabric against Gram-negative bacteria, *Escherichia coli* at 10^{-4} dilution and Figure 7 shows the zone of inhibition of the treated fabric at 10% concentration against *Escherichia coli*.

Table 3: Zone of Inhibition using *Escherichia coli*.

E.Coli Dilution	**Zone of Inhibition (mm)**					
	Control	**5%**	**10%**	**15%**	**20%**	**25%**
1×10^{-4}	0.8	3.1	5.2	3.4	5.8	4.2
1×10^{-5}	0.8	3.3	6.1	3.8	4.8	5.3
1×10^{-6}	0.9	6.7	7.1	9.5	10.1	6.2

The relationship between the chemical concentration and bacterial concentration against Gram-positive *Escherichia coli* is given by:

for 10^{-4} dilution:

$$y = -0.3151x^2 + 2.9055x - 1.3656 \qquad \text{------(5)}$$
$$R^2 = 0.7021$$

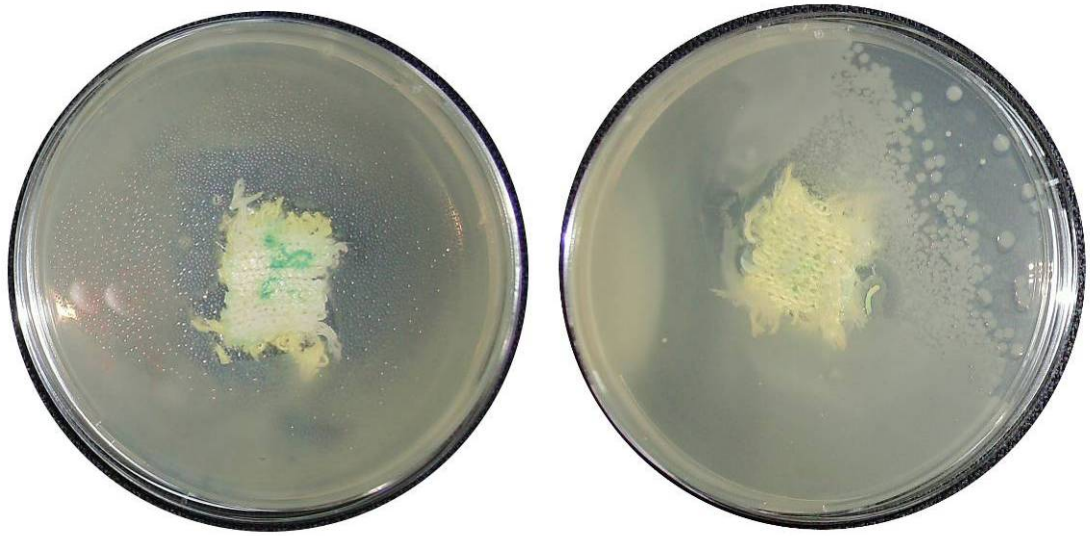
for 10^{-5} dilution:

$$y = -0.8414x^2 + 7.0095x - 5.0297 \qquad \text{------(6)}$$
$$R^2 = 0.9155$$

for 10^{-6} dilution:

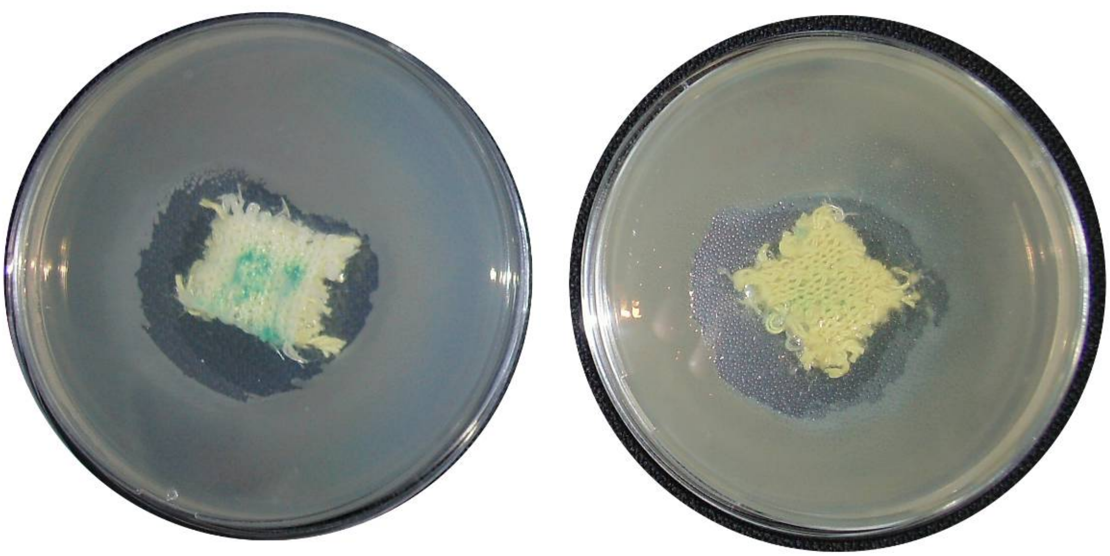
$$y = -0.3283x^2 + 2.9624x - 1.6362 \qquad \text{------(7)}$$
$$R^2 = 0.7441$$

Once again, the effect of chemicals against the Gram-negative bacteria shows the optimum antimicrobial activity, with zones of 5.2mm, 6.1mm and 7.1mm, at 10% concentration of the antibacterial recipe.



White face of the fabric Yellow face of the fabric

Figure 6: Zone of inhibition of control untreated fabric against *Escherichia coli*



White face of the fabric Yellow face of the fabric

Figure 7: Zone of inhibition of treated fabric (10% recipe) against *Escherichia coli*

Durability of Antimicrobial Finish against Gram-positive Bacteria

The novel slash resistant fabric is made of two layers with different yarn constituents, one face with Kevlar that is yellow in colour, and the other face with the composite WF 528 that is white in colour. Both yarns have different absorption properties and hence the durability of the antimicrobial finish was evaluated on both the faces of the fabric.

Table 4 and Figure 8 show the zone of inhibition in mm against *Staphylococcus aureus* after 1, 5 and 10 washes, with and without a cross-linking agent (CL), with Kevlar (Yellow) as the test face. The reduction in zone of inhibition after each wash is expressed as a percentage in Table 4 and is shown graphically in Figure 9.

Table 4: Durability of Antimicrobial Activity on Yellow (Kevlar) Face with and without Cross-linking Agent (CL) against *Staphylococcus aureus.*

S.Aureus Dilution	Yellow Face - with CL							Yellow Face - without CL						
	Treated Unwashed Control	1 Wash		5 Wash		10 Wash		Treated Unwashed Control	1 Wash		5 Wash		10 Wash	
		Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)		Zone of Inhibit ion (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)
10^{-4}	6.7	4.2	37%	1.0	85%	1.6	76%	5.8	3.2	45%	2.2	62%	2.0	66%
10^{-5}	6.4	3.1	52%	2.0	69%	2.6	59%	4.8	1.5	69%	0.6	88%	0.5	90%
10^{-6}	6.6	3.2	52%	2.2	67%	3.5	47%	10.2	3.5	66%	1.8	82%	0.0	100%

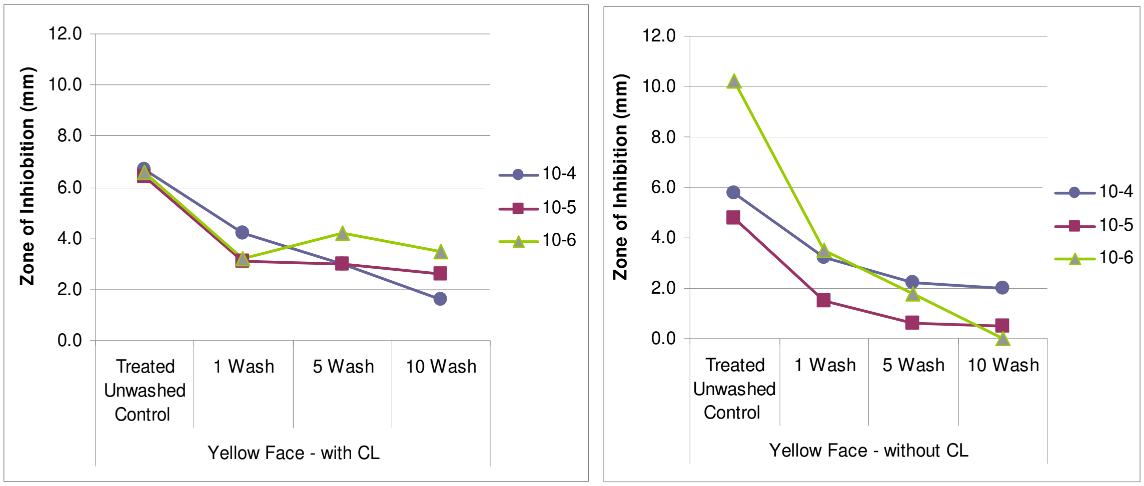


Figure 8: Durability of antimicrobial activity on yellow (Kevlar) face with and without cross-linking agent (CL) against *Staphylococcus aureus*

It can be seen from Table 4 that the percent reduction in zone of inhibition after washing is mostly higher in the case of samples without the cross-linking agent. The average reduction at all dilutions is 85% after 10 washes for the specimen without the cross-linking agent while the average reduction for the specimen treated with the cross-linking agent is only 60%. For a bacterial dilution to the order of 10^{-6}, the percentage reduction in zone of inhibition is 47% after 10 washes with the cross-linking agent and there is no antimicrobial activity at all without the cross-linking agent.

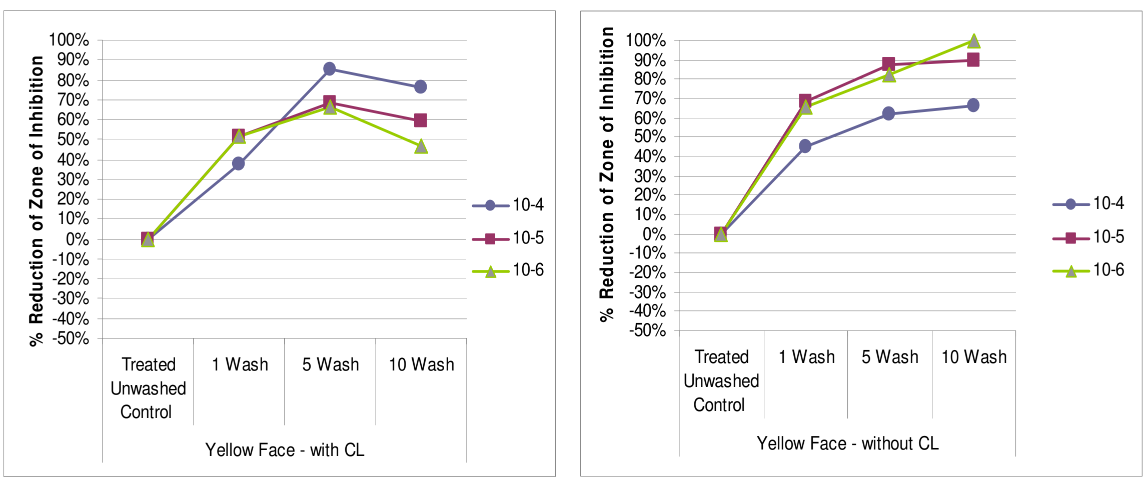


Figure 9: Percent reduction in zone of inhibition on yellow (Kevlar) face with and without cross-linking agent (CL) against *Staphylococcus aureus*

Comparing with the antimicrobial activity on the yellow Kevlar face, the white WF 528 face shows a higher retention of the antimicrobial agents when a cross-linking agent is used. The average reduction at all dilutions in the zone of inhibition is 37% for the white face whereas it is 60% for the yellow Kevlar face (see Table 5 and Figure 11). For treatments without the cross-linking agents, the percentage reduction in zone of inhibition is very similar at 85% and 82% for yellow face and white face respectively.

Table 5: Durability of Antimicrobial Activity on White (WF 528) Face with and without Cross-linking Agent (CL) against *Staphylococcus aureus*.

S.Aureus Dilution	White Face - with CL							White Face - without CL						
	Treated Unwashed Control	1 Wash		5 Wash		10 Wash		Treated Unwashed Control	1 Wash		5 Wash		10 Wash	
		Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)		Zone of Inhibit ion (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)
10^{-4}	4.6	6.0	-30%	6.2	-35%	4.0	13%	5.2	5.3	-2%	2.9	44%	2.0	62%
10^{-5}	4.7	3.1	34%	3.6	23%	2.2	53%	11.6	5.9	49%	2.9	75%	1.9	84%
10^{-6}	7.7	5.5	29%	7.0	9%	4.1	47%	11.2	10.2	9%	4.4	61%	0.0	100%

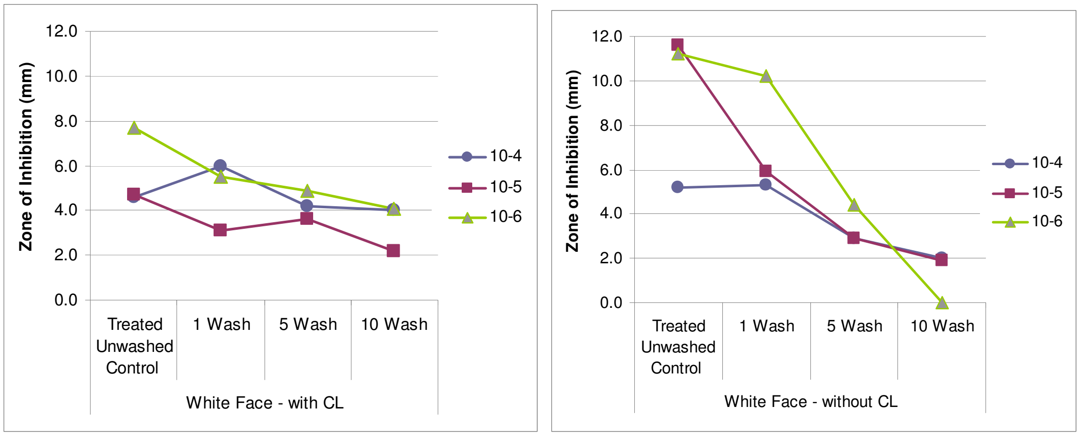


Figure 10: Durability of antimicrobial activity on white (WF 528) face with and without cross-linking agent (CL) against *Staphylococcus aureus*

The antimicrobial activity of a fabric treated with an antibacterial agent is generally expected to either stay the same or to reduce after every single wash. But, during this experiment, for bacterial dilutions to the order of 10^{-4}, there was an increase of 30% in the antimicrobial activity after 1 and 5 washes. This could be due to experimental error or there could have been a higher take-up of the antimicrobial chemicals at the particular part of the specimen.

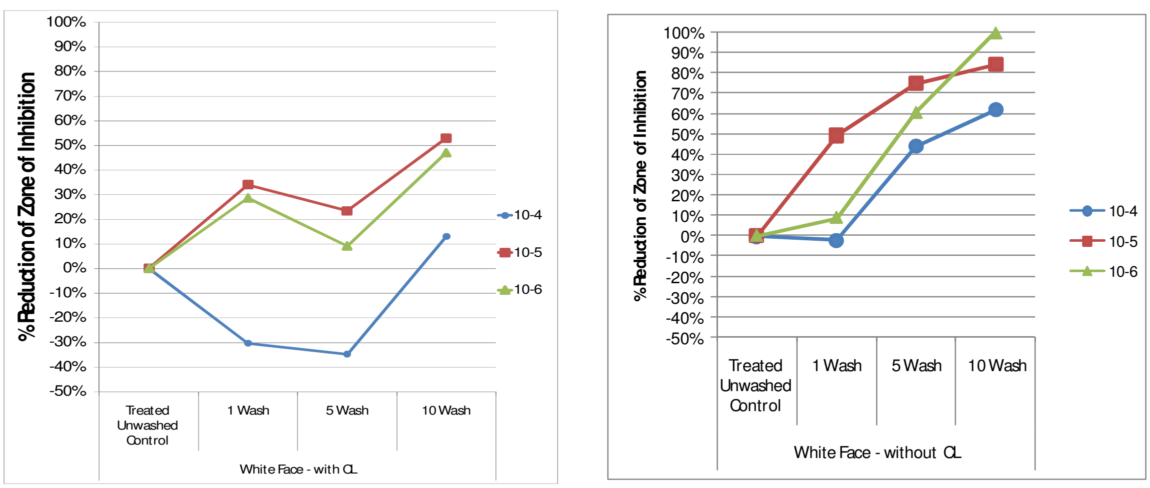


Figure 11: Percent reduction in zone of inhibition on white (WF 528) face with and without cross-linking agent (CL) against *Staphylococcus aureus*

Durability of Antimicrobial Finish against Gram-negative Bacteria

The durability of the antimicrobial finish on Gram-negative bacteria is lower when compared to the durability against Gram-positive bacteria. The reduction in antimicrobial activity increased to 100% on both faces of the fabric, with and without the cross-linking agent for bacterial dilutions in the order of 10^{-6} even though higher concentrations of bacteria showed some antimicrobial activity.

Table 6: Durability of Antimicrobial Activity on Yellow (Kevlar) Face with and without Cross-linking Agent (CL) against *Escherichia coli*.

E.Coli Dilution	Yellow Face - with CL							Yellow Face - without CL						
	Treated Unwashed Control	1 Wash		5 Wash		10 Wash		Treated Unwashed Control	1 Wash		5 Wash		10 Wash	
		Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)		Zone of Inhibit ion (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)
10^{-4}	7.2	3.0	58%	1.2	83%	1.4	81%	3.2	3.9	-22%	0.0	100%	1.1	66%
10^{-5}	8.0	6.7	16%	2.4	70%	1.3	84%	11.0	5.3	52%	0.0	100%	1.0	91%
10^{-6}	22.0	9.9	55%	2.3	90%	0.0	100%	8.0	5.9	26%	3.7	54%	0.0	100%

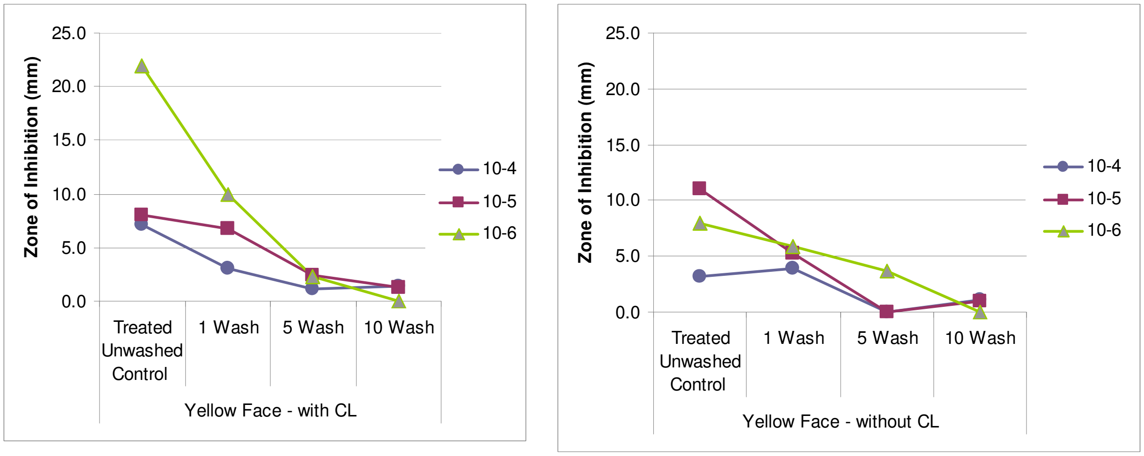


Figure 12: Durability of antimicrobial activity on yellow (Kevlar) face with and without cross-linking agent (CL) against *Escherichia coli*

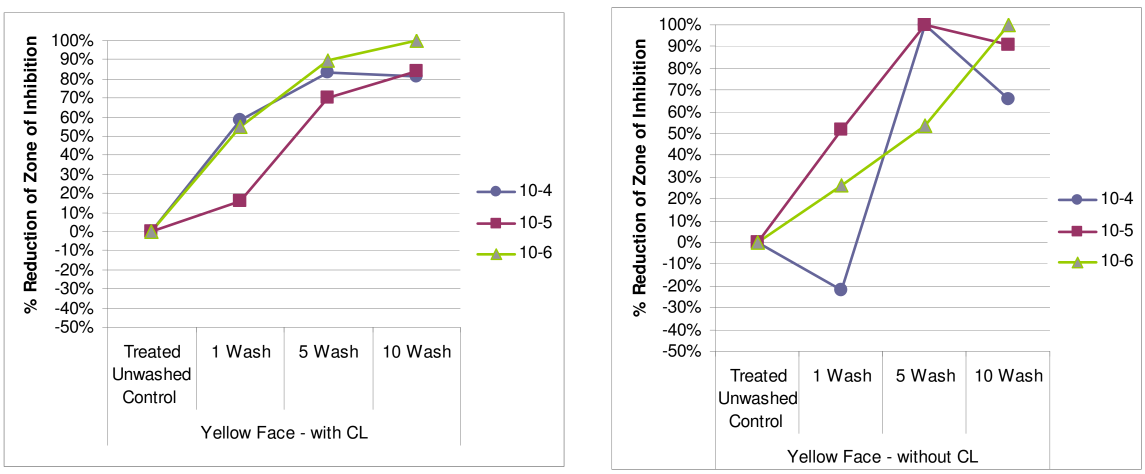


Figure 13: Percent reduction in zone of inhibition on yellow (Kevlar) face with and without cross-linking agent (CL) against *Escherichia coli*

There was no significant difference in the retention of the antimicrobial agents on the yellow face when a cross-linking agent is used. The average percentage reduction in zone of inhibition is 88% with cross-linking agent and 86% without cross-linking agent (see Table 6).

Table 7: Durability of Antimicrobial Activity on White (WF 528) Face with and without Cross-linking Agent (CL) against *Escherichia coli*.

E.Coli Dilution	White Face - with CL							White Face - without CL						
	Treated Unwashed Control	1 Wash		5 Wash		10 Wash		Treated Unwashed Control	1 Wash		5 Wash		10 Wash	
		Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)		Zone of Inhibit ion (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)	Zone of Inhibition (mm)	Reduction in Zone of Inhibition (%)
10^{-4}	6.1	6.4	-5%	4.7	23%	4.2	31%	3.3	4.7	-42%	0.0	100%	1.0	70%
10^{-5}	11.5	6.6	43%	5.2	55%	3.7	68%	11.0	4.9	55%	0.0	100%	1.5	86%
10^{-6}	14.8	9.7	34%	5.8	61%	0.0	100%	9.0	9.0	0%	7.6	16%	0.0	100%

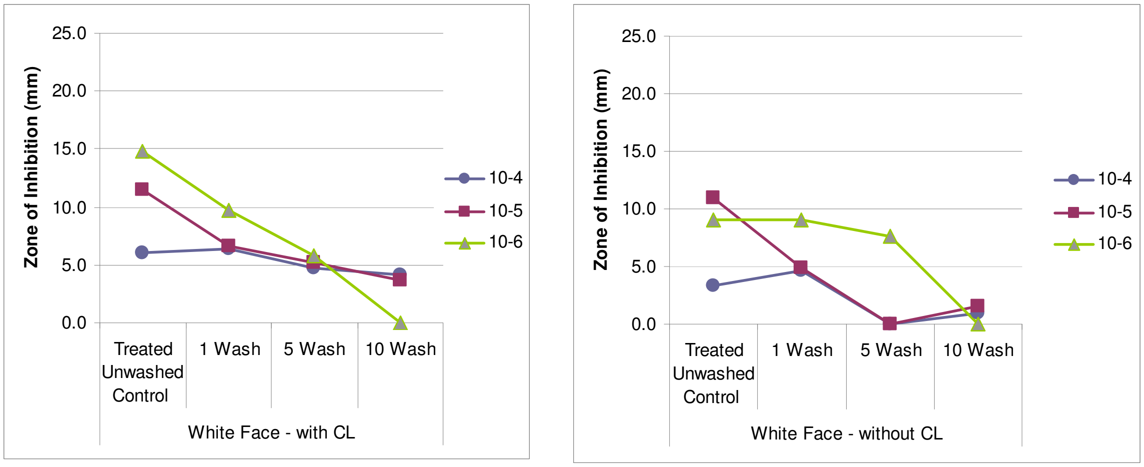


Figure 14: Durability of antimicrobial activity on white (WF 528) face with and without cross-linking agent (CL) against *Escherichia coli*

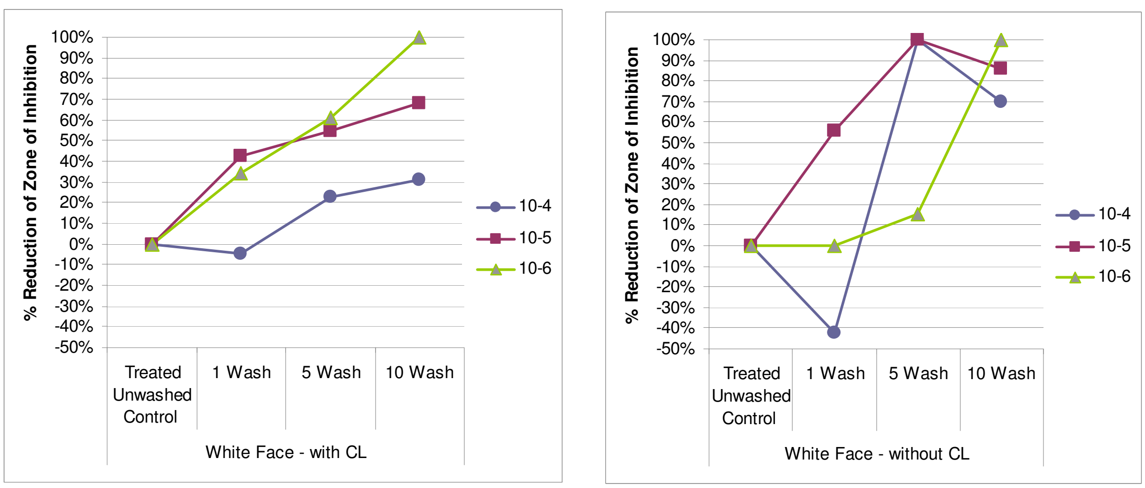


Figure 15: Percent reduction in zone of inhibition, on white (WF 528) face with and without cross-linking agent (CL) against *Escherichia coli*

Compared to the antimicrobial activity on the yellow Kevlar face, there is an increase in activity by 11% on the white WF528 face when a cross-linking agent is used indicating the durability of the antimicrobial.

CONCLUSIONS

One of the main objectives of this research programme is to incorporate suitable antimicrobial agents onto the novel two-layered weft knitted slash resistant fabric. In this study, a synergistic system of chemical formulation has been developed to impart antibacterial activities to the novel slash resistant fabric that has just 13.6% by weight of fibres available as candidate for this activity. The treated fabric was tested against both

Gram-positive and Gram-negative bacteria. The antimicrobial activity of the treated slash resistant fabric was studied at different concentrations of chemical formulation and it was found that optimum antimicrobial activity is reached at 10% concentration and, thereafter, the increase is not significant.

The durability of the chemical formulation at 10% concentration has been studied for up to 10 washes. The study indicates that the antimicrobial activity existed after testing up to 10 washes. A comparative study has been conducted on the durability by adding a cross-linking agent into the antimicrobial chemical formulation and it was found that the percentage retention of the antimicrobial agent is significantly higher on the white WF528 face when a cross-linking agent is used.

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