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Influence of Raw Composition of Plain Plated Knits on their Antimicrobial Characteristics

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Abstract
This paper deals with the determination of the antimicrobial efficiency of plain plated weft knits antimicrobial treated with commercial silver chloride (AgCl (iSys Ag, Germany)) dispersed in a reactive organic-inorganic binder (iSys MTX, Germany). Antimicrobial treatment was performed by the exhaustion method. The morphology of untreated and antimicrobial treated knits was analysed using Scanning Electron Microscopy (SEM) to confirm the presence of silver particles on the fibre surface. The antimicrobial activity was estimated using the Agar diffusion Test (according to standard EN ISO 20645:2004 (qualitative method)) and antibacterial finishes in a Textile Materials Test (according to the AATCC 100–1999 standard (quantitative method)). Two strains of bacteria - E. coli and S. aureus were used in this research. The results obtained proved that the fibre type and raw composition have an important influence on the relationship between the fibre surface and antimicrobial effect of the material formation and optimal antimicrobial efficiency obtained. It was found that pure fibre cotton and man-made bamboo knits had the highest antimicrobial activity for both types of bacteria (E. coli and S. aureus). Meanwhile knits of 100% polyester fibre and blended knits containing polyester fibre had an insufficient effect on the bacterial growth. It was also established that antimicrobial treated knits with the same fibre composition and very similar total linear density of folded yarns but with a different number of folded yarns in the structure have different antimicrobial activity. This is because the higher number of folded yarns in the knitted loop has a larger surface area and, herewith, larger antimicrobial acting area.

Key words: *antimicrobial treatment, antimicrobial activity, plain plated knits.*

Introduction

Antimicrobial textile is one of the fastest growing areas of multifunctional textiles. Antimicrobial fabrics are important not only in medical applications but also in terms of daily life usage (everyday sports and leisure wear, socks and underwear, etc.). The application of antimicrobial finishes to textiles can prevent bacterial growth on textiles [1].

Microorganisms are so small that they are microscopic (invisible to the naked eye). Microorganisms i.e. bacteria, fungi, mildew, mould and yeast, are found everywhere in nature, even in hostile environments. The human skin is usually crowded with innumerable microorganisms. A suitable temperature, moisture, dust and receptive surface provide perfect conditions for their growth [2]. The growth of microorganisms on textiles inflicts a range of unwanted effects not only on the textile itself but also on the wearer [3]. Antimicrobial treatment for textile materials is necessary to fulfil the following objectives: to avoid cross infection by pathogenic microorganisms, to control infestation by microbes, to arrest the metabolism in microbes in order to reduce the formation's odour; and to safeguard textile products from staining, discoloration and quality deterioration [4].

Despite the growth of antimicrobial textile usage, we are still facing a number of problems such as the durability of antimicrobial properties in the product exploitation period and the adaptation of microorganisms for chemical use. Therefore in order to produce a product with antimicrobial properties proper orientation is required, especially in a market of numerous fibres and various chemicals providing antimicrobial properties, as well as a properly selected method of application of antimicrobial agents on textiles [5].

The most important antimicrobial substances used in textile finishing are organic (biguanide, izotiazoline, ammonium compounds with organosilicone etc.), inorganic (metal ions (Cu, Ag, Zn), zeolites, ceramic substrates with metal ions etc.) chemicals and chemicals of natural origin (chitosan, polysaccharides from clams, sea, some dumb, plant extracts, etc.). Silver nanoparticles (AgNPs), silver nitrate ($AgNO_3$), silver chloride AgCl compounds are the best known and most commonly used inorganic antimicrobial finishes agents/materials [6, 7]. Unfortunately many of these agents have a possible harmful or toxic effect. Nanosilver, on the other hand, is a relatively non-toxic disinfectant that can significantly reduce many strains of bacteria and fungi [8]. Silver nanoparticles have some unique chemical and physical properties such as a high surface area, small size (< 20 nm) and high dispersion, which make them an effective antimicrobial agent [9].

Antimicrobial agents can be applied to the textile substrate by exhaust, pad-dry-cure, coating, spray and foam techniques [10, 11]. A well-known technique for textile modification is the sol-gel method, which allows the functionalisation of textiles in a wide range of applications [9, 12]. Sol-gel has been extensively explored for applications such as coating. It is claimed that sol-gel technology can enable the coating of textiles with almost unlimited functionality by incorporating functional agents into sol-gel nanoparticles [3].

In practice a wide variety of methods are used to assess the bactericidal, bacteriostatic and antifungal properties of textile materials in qualitative and quantitative aspects. Relatively shorter and simpler qualitative detection methods are those which determine if textile material has antimicrobial properties. Meanwhile the other and longer-lasting methodology allows quantification of antimicrobial agents' "strength" [13].

A large number of studies have been performed in order to investigate the resistance of various spectrum antimicrobial materials on textiles. Many researchers have analysed antimicrobial activity after antimicrobial treatment of woven fabrics with cotton, wool, polyamide, polyester threads and combinations thereof, and non-woven materials with viscose polyamide/polypropylene and polyester

Table 1. Composition of plain plated knits.

Group	Variant of knits	Raw material and linear density of ground yarn and plating yarn	Fibre composition, %	Total linear density, tex
I	C1	Cotton 29.4 tex + cotton 29.4 tex	100	58.8
	C2	Cotton 14.8 tex × 2 + cotton 14.8 tex × 2		59.2
	B1	Man-made bamboo 29.4 tex + man-made bamboo 29.4 tex		58.8
	B2	Man-made bamboo 14.8 tex × 2+ man-made bamboo 14.8 tex × 2		59.2
	PES3	Polyester 25 tex + polyester 25 tex		50.0
II	C1PA	Cotton 29.4 tex + polyamide 10 tex × 2	60 / 40	49.4
	C2PA	Cotton 14.8 tex × 2 + polyamide 10 tex × 2		49.6
	B1PA	Man-made bamboo 29.4 tex + polyamide 10 tex × 2		49.4
	B2PA	Man-made bamboo 14.8 tex × 2 + polyamide 10 tex × 2		49.6
	PES3PA	Polyester 25 tex + polyamide 10 tex × 2	56 / 44	45.0
III	C1PES	Cotton 29.4 tex + polyester 20 tex	60 / 40	49.4
	C2PES	Cotton 14.8 tex × 2 + polyester 20 tex		49.6
	B1PES	Man-made bamboo 29.4 tex + polyester 20 tex		49.4
	B2PES	Man-made bamboo 29.4 tex + polyester 20 tex		49.6
	PES3PES	Polyester 25 tex + polyester 20 tex	100	45.0

fibres [14–20]. K. Erdem and S. Yurudu investigated the antimicrobial activity of woven fabrics after several cycles of washes, which were treated with Dimethyltetradecyl Ammonium Chloride [1]. However, there were only a few studies found in which the antimicrobial activity of antimicrobially treated knits is researched.

The main goal of this research was to investigate and evaluate the antimicrobial activity in pure fibre and blended fibre knits after treatment with commercial antimicrobial agents iSys Ag and iSys MTX (CHT, Germany) by both qualitative and quantitative methods and to establish the influence of the fibre composition in the knit on the antimicrobial activity of antimicrobially treated knits.

This study gives essential information such as the effect of the fibre composition of plain plated pure and blended fibre knits on antimicrobial activity due to antimicrobial materials “stick/cling/coating” to the surface of the fibre.

Materials and methods

Experimental samples were knitted in a plain plated pattern on a circular 14E gauge single needle-bed weft knitting machine. There were three groups of samples made for this research. In each group there were five variants of knits – two variants with cotton, two with man-made bamboo, and one with polyester plating yarn. The ground yarns used for the knitting of samples of I group were cotton, man-made bamboo and polyester (the same yarn as the plating one). The ground yarn used for the knitting of samples of II group was polyamide, and for the samples of III group – polyester thread. The composition of knitted samples is presented in ***Table 1***.

Antimicrobial treatment

Silver nanoparticles in AgCl from iSys Ag (CHT, Germany) were used as an antimicrobial agent in combination with organic-inorganic binder iSys MTX (CHT, Germany). Antimicrobial substances/agents on the textile materials were covered using the exhaust method. The main conditions of antimicrobial treatment were as follows: aqueous solution of 25 °C, 2 g/l of iSys AG and 10 g/l of iSys MTX, pH 5.5, and duration 10 min. After that, all samples were centrifuged for 12 min at 1200 r.p.m, tumble dried, and permanently treated for 1 min at 160 °C.

Analysis of fibre surfaces using SEM microscopy

Before and after antimicrobial treatment, i.e. after samples had been coated with a thin sol-gel layer, the fibre surface was scanned and analysed by SEM (Scanning Electron Microscopy) Quanta 200 FEG, FEI, Netherlands (20 kV, magnification range of specimen - 5000×).

Assessment of antimicrobial activity

The antimicrobial efficiency of samples was tested with Gram-negative bacteria *E. coli* (KMY1T) and Gram-positive bacteria *S. aureus* (ATCC25923). These bacteria were chosen as being the microorganisms most frequently taken from skin wounds on humans [18].

Evaluation of the antimicrobial efficiency was carried out by two methods: in accordance with the EN ISO 20645:2004 (Agar diffusion plate test (qualitative)) and AATCC 100–1999 standards (modified method), which allow to evaluate the activity of antimicrobial finishing in a quantitative dimension.

The qualitative antimicrobial activity of the samples investigated was estimated using the Agar diffusion plate test (ISO20645:2004) method. To guarantee contact of the specimens of knits with the agar inoculated with bacteria, sterilised glass rings were used. The plates were incubated for 18 h at 37 °C and afterwards the width of the inhibition zone was calculated:

$$H = (D - d)/2 \qquad (1)$$

where, H is the inhibition zone in mm, D the total diameter of the sample and inhibition zone in mm, and d the diameter of sample in mm.

Evaluation criteria of the antimicrobial effect:

- ≥ 1 mm inhibition zones and no growth under the specimen are accepted as a good effect;
- 0 mm inhibition zone and slight growth are evaluated as a limited effect;
- 0 mm inhibition zone and moderate or heavy growth under the sample is evaluated as an insufficient effect.

The quantitative antimicrobial activity of the knits was estimated for Gram-positive bacteria *S. aureus* (ATCC25923) and Gram-negative bacteria *E. coli* (KMY1T) according to the AATCC 100–1999 standard method. Cultures of bacteria were grown in broth LB medium at 37 °C overnight, which were then transferred into a nutrient broth at an initial optical density (OD) of 0.1 at 600 nm and allowed to grow at 37 °C. When the cultures reached an optical density of 0.6 OD at 600 nm, the samples of knits (4 cm^2 in size) were put into the culture suspension. Bacterial suspensions with the knits were incubated at 37 °C until the optical density reached 1.0 OD. Afterwards samples of 50 µl each were taken, diluted with saline and then transferred onto nutrient agar plates. The plates were allowed to grow for 15 h

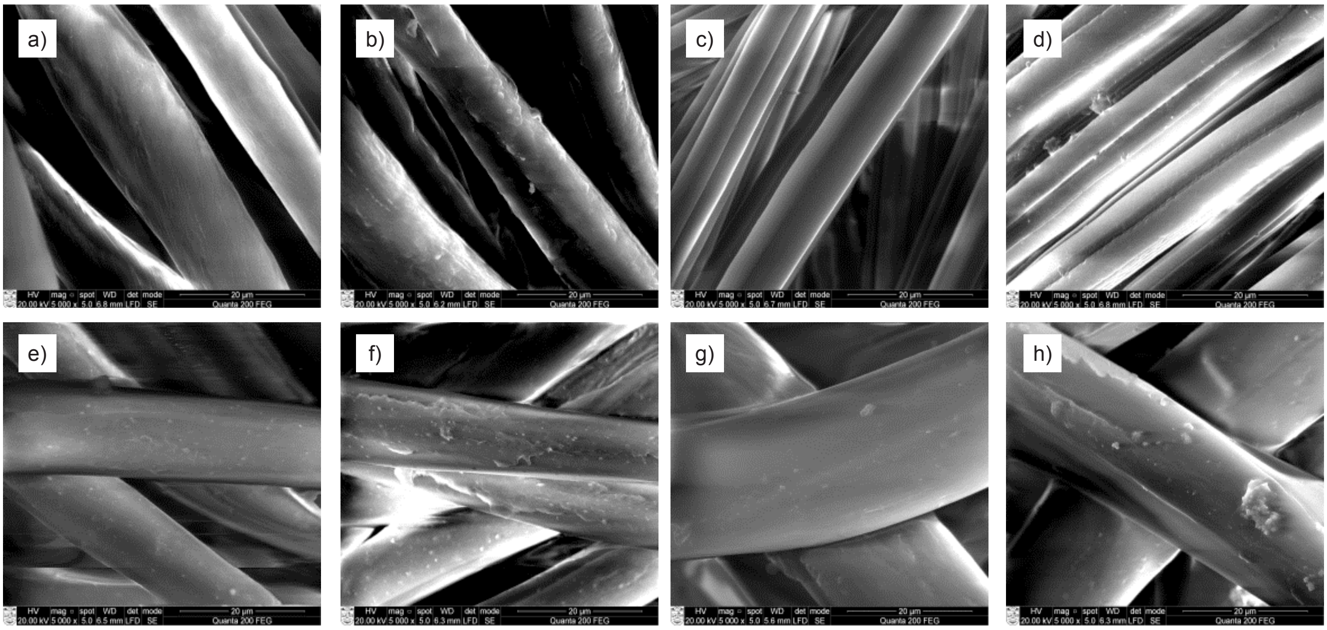

Figure 1. *SEM images of untreated and treated fibres: a) untreated C1 (cotton), b) treated C1 (cotton), c) untreated B1 (man-made bamboo), d) treated B1 (man-made bamboo), e) untreated PES3 (polyester), f) treated PES3 (polyester), g) untreated PA (polyamide), h) treated PA (polyamide). The magnification range is 5000×.*

at 37 °C and then bacteria counted. To evaluate antimicrobial activities of the treated knits, the reduction in the colony number between the treated and untreated knits after incubation was determined. The percentage reduction was calculated as follows:

$$R = (B - A)/B \qquad (2)$$

where, R is the percentage reduction of bacteria in %, and B & A the numbers of colony-forming units (CFU ml^{-1}) recovered from the untreated and treated knits, respectively, after inoculation and incubation.

Results and discussions

Surface analysis of the untreated and antimicrobial treated knitted samples was performed on purpose to determine changes caused by the treatment with an antimicrobial substance (iSys AG and iSys MTX). In addition, scanning electron microscopy can prove the presence of silver nanoparticles and their distribution on the fibre surface. Fibre surface images of untreated and antimicrobial treated knits are presented in ***Figure 1***.

In the SEM images, the appearance of the cotton, man-made bamboo, polyamide and polyester fibre surface before the treatment of knits with an antimicrobial substance compared with the same knits after treatment with the AgCl based agent deposited by the deposition process is presented. In all images of knitted samples treated with the antimicrobial agent, silver nanoparticles are visible more or less distributed over the surface of the fibres, indicating that the antimicrobial process resulted in the successful deposition of the agent onto the fibres. Also visible are additional three-dimensional irregularities on the surface that can be correlated with the ingredients of the dispersion of the antimicrobial AgCl based agents.

In this research work, the antimicrobial activity of antimicrobial treated knits was investigated using two methods. The results of the test of antimicrobial activity according to the EN ISO 20645:2004 (Agar diffusion plate test) standard with two bacterial species (*Escherichia coli* and *Staphyloccoccus aureus*) are presented in ***Table 2***.

Table 2. *Results of antimicrobial activity according to Standard EN ISO 20645:2004 (qualitative evaluation); a) face and b) reverse side of the knit.*

Variant of knits	Inhibition zone, mm/Growth/Assessment	
	Escherichia coli	*Staphylococcus aureus*
C1	1.5/none/good effect	1/none/good effect
C2	2/none/good effect	1/none/good effect
B1	1/none/good effect	0/slight/limit of efficacy
B2	1/none/good effect	0/slight/limit of efficacy
PES3	0/moderate/insufficient effect	0/heavy/insufficient effect
C1PA	(0/none/good effect)[a]/(0/slight/limit of efficacy)[b]	(0/none/good effect)[a]/(0/moderate/ insufficient effect)[b]
C2PA	(0/none/good effect)[a]/(0/slight/limit of efficacy)[b]	(0/none/good effect)[a]/(0/moderate/ insufficient effect)[b]
B1PA	(0/none/good effect)[a]/(0/slight/limit of efficacy)[b]	(0/slight/limit of efficacy)[a]/(0/moderate/ insufficient effect)[b]
B2PA	(0/none/good effect)[a]/(0/slight/limit of efficacy)[b]	(0/slight/limit of efficacy)[a]/(0/moderate/ insufficient effect)[b]
PES3PA	(0/moderate/insufficient effect)[a] /(0/ moderate/insufficient effect)[b]	(0/heavy/insufficient effect)[a]/(0/moderate/ insufficient effect)[b]
C1PES	(0/none/good effect)[a]/(0/heavy/insufficient effect)[b]	(0/none/good effect)[a]/(0/heavy/insufficient effect)[b]
C2PES	(0/none/good effect)[a]/(0/heavy/insufficient effect)[b]	(0/none/good effect)[a]/(0/heavy/insufficient effect)[b]
B1PES	(0/none/good effect)[a]/(0/heavy/insufficient effect)[b]	(0/slight/limit efficacy)[a]/(0/heavy/insufficient effect)[b]
B2PES	(0/none/good effect)[a]/(0/heavy/insufficient effect)[b]	(0/slight/limit of efficacy)[a]/(0/heavy/ insufficient effect)[b]
PES3PES	(0/heavy/insufficient effect)[a] /(0/heavy/ insufficient effect)[b]	(0/heavy/insufficient effect)[a]/(0/heavy/ insufficient effect)[b]

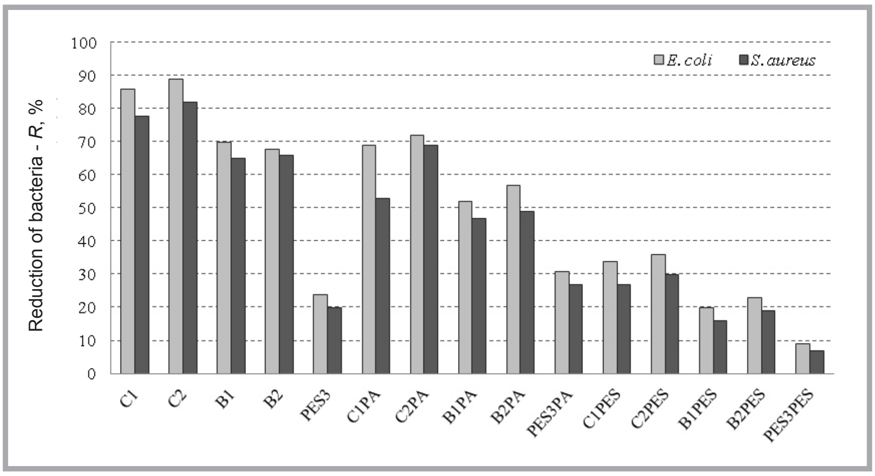

Figure 2. *Bacterial reduction R (%) of E. coli and S. aureus bacteria determined according to the AATCC 100–1999 method.*

The results in ***Table 2*** demonstrate that pure cotton fibre plain plated knits C1 and C2 were characterized by a good antimicrobial effect against Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria (wherein the inhibition zone with *E. coli* was 1.5 - 2 mm and with Gram-positive bacteria *S. aureus* – 1 mm). Very similar results of antimicrobial activity after antimicrobial treatment (in which the same antimicrobial agents were used as in this research) were obtained by B. Tomšič et al. [14, 21]. It is known that natural cellulose fibres possess abundant hydroxyl groups on their surface and provide a suitable substrate for metal oxide deposition. A positive antimicrobial effect against *E. col*i bacteria was also obtained for 100% man-made bamboo knits, whereas only a marginal effect against *S. aureus* bacteria was observed (no inhibition zone, some bacterial colonies were found on the sample but the growth had been suspended). For pure polyester fibre knit PES3 opposite results were obtained. In this case, the antimicrobial effect was not altogether determined.

By using a standard method to identify the antimicrobial activity, it is difficult to evaluate objectively to what extent antimicrobial treated blended plain plated knits affect specific/selected bacteria. Investigating plated knits, the antimicrobial effect was determined separately for both sides of the knit because in the plain plated knit the face side was structured from plating yarns and the reverse side – from ground yarns. The results obtained show that the natural cotton fibres and man-made bamboo fibres on the face side of the blended knits (in groups II and III) affect negatively the growth of bacteria *E. coli* and *S. aureus*; however, the inhibition zone was not identified, even though the growth of bacteria on the sample was intercepted or only several colonies occurred. Whereas different results were obtained for the reverse side of the blended plain plated knits, that is structured of synthetic threads (in group II – polyamide threads, in group III – polyester threads). SEM images (***Figure 1***) demonstrate that antimicrobial substance Isys AG (silver nanoparticles) and/or iSys MTX (organic-inorganic binder) cover the surface of the fibres. However, as the results obtained demonstrate, polyamide threads on the reverse side of the antimicrobial treated knits in group II, tested with *E. coli* bacteria, have only marginal effectiveness and the antimicrobial effect with *S. aureus* bacteria was not observed in all. Meanwhile on the polyester threads (on the reverse side of the knits in group III) no antimicrobial activity against both types of bacteria was observed. These results indicate that the interaction of synthetic fibres with the organic-inorganic binder is adsorptive. The organic-inorganic binder is adsorbed on the fibre surface (antimicrobial agent comes into contact with the fibre surface), but there is no chemical interaction or it is weak as it is effected by physical interaction.

Qualitative analysis of the results of the antimicrobial effectiveness of blended plain plated knits processed by a commercial antimicrobial agent cannot precisely evaluate whether such a knit will negatively affect the growth of the bacteria, especially if a different antimicrobial effect has been found on the opposite sides of the knit.

Quantitative results of the antimicrobial activity of the knits were obtained at the accredited National Public Health Surveillance Laboratory (Kaunas, Lithuania) using the agar diffusion test. In order to evaluate the antimicrobial properties of pure fibre and blended fibre plain plated knits treated by antimicrobial agents more accurately, the Antibacterial Finishes on Textile Materials Test method was used, which allows to quantitatively evaluate the resistance of antimicrobial processed knits to the growth of microorganisms. By employing this method, the general antimicrobial activity of the knit (the reverse side as well as the face side) is evaluated since the whole knit is worked into a bacterial growth medium. The results of bacterial reduction of knits coated with a silver and organic-inorganic binder are presented in ***Figures 2*** and ***3***.

The Ag particles on the treated knitted samples facilitated bacterial reduction to a greater or lesser extent, which strongly depends not only on the concentration of the Ag adsorbed but also on the nature of the fibres. Analysis of the test results indicates that the antimicrobial activity of the treated knits was higher against Gram-negative (*E. coli*) bacteria than Gram-positive (*S. aureus*) bacteria. However, all that matters is that pure cotton knits exhibited a substantially greater bacterial reduction (up to 86% against *E. coli* and up to 78% against *S. aureus*, (***Figure 3.a***)) than the other plated knits investigated, even more than man-made bamboo knits (up to 70% against *E. coli* and up to 65% against *S. aureus*). Meanwhile PES3, PES3PA and blended knits containing polyester fibres had an insufficient effect on the bacterial growth (***Figure 3.d***). The reduction in *E. coli* bacteria on these knits was from 9 to 36%, and for *S. aureus* bacteria – from 7 to 30%.

The results demonstrate that the growth of bacteria on the PA knitted fabrics plated with cotton and man-made bamboo yarns (in group II) was 11 - 18% less with *E. coli* bacteria and 13 - 25% less with *S. aureus* bacteria compared with the pure fibre knits (C1, C2, B1, and B2). Meanwhile the difference in antimicrobial activity of PES knits plated with cotton and man-made bamboo yarns (in group III) was 45% less with *E. coli* and 53% less with *S. aureus* bacteria compared to pure fibre knits. The low capacity of PA and PES fibres to adsorb the Ag compound can be explained by their high hydrophobicity and crystallinity, which hindered access to the aqueous solution

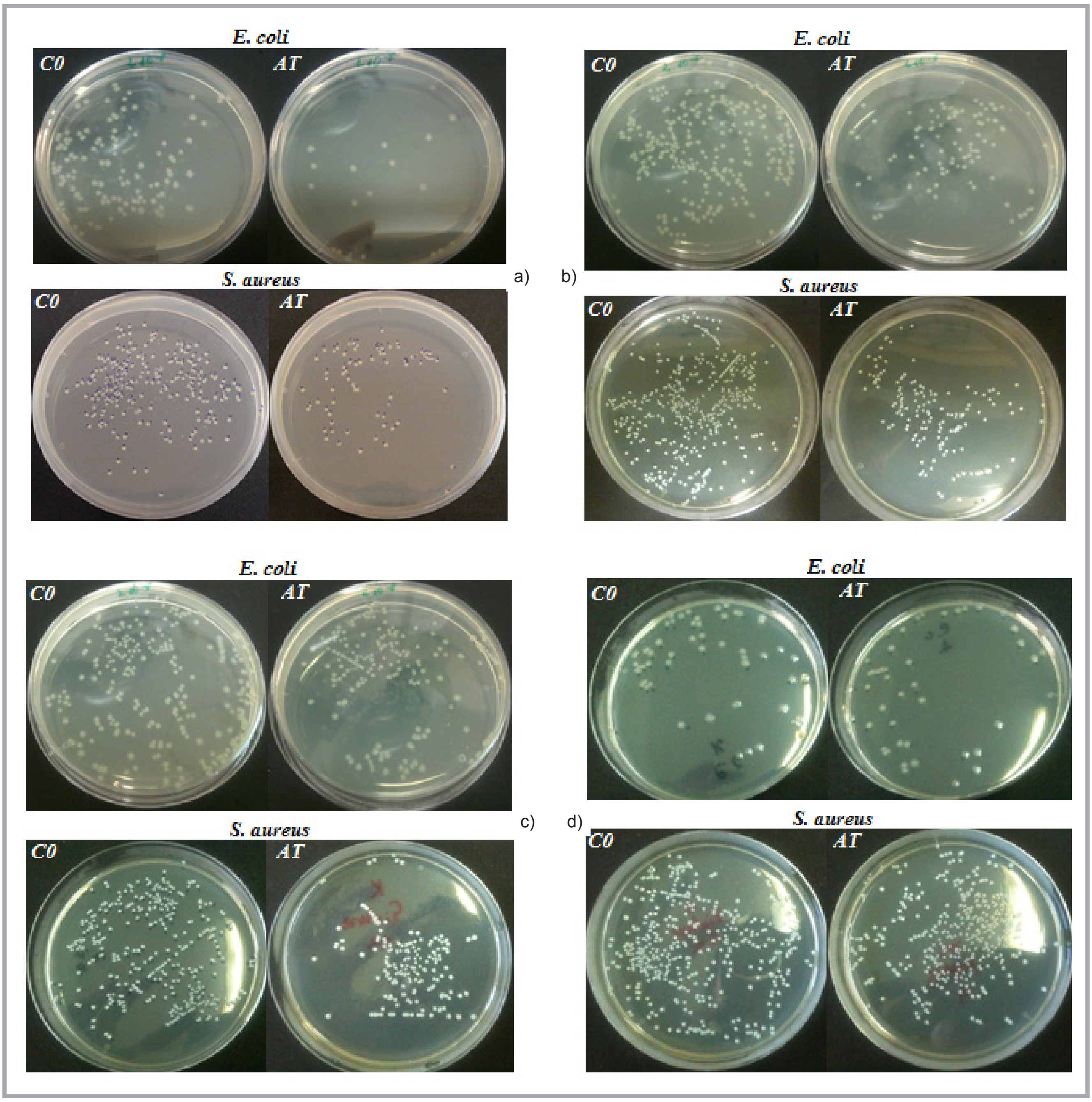

Figure 3. *Antimicrobial activity of knitted samples investigated against E. coli and S. aureus by the AATCC 100–1999 standard method (quantitative evaluation): a) C1 (cotton), b) C1PA (cotton+polyamide), c) C1PES (cotton+polyester), d) PES3PES (polyester).* ***Note****: C0- control sample, AT- antimicrobial treated sample.*

and, consequently, Ag adsorption from the finishing bath.

In ***Figure 2***, the results also demonstrate the dependence of the antimicrobial activity of the knits on the yarn construction. It is evident that antimicrobial treated knits with the same fibre composition and very similar total linear density of yarns but with a different number of folded yarns in the structure have different antimicrobial activity. This is because the higher number of folded yarns in the knitted loop has a larger surface area and, herewith, larger antimicrobial acting area. These differences in antimicrobial activity are not high, only a few percent, however they are apparent and consistent.

Conclusion

After antimicrobial treatment with silver nanoparticles in AgCl from iSys Ag (CHT, Germany) in combination with organic-inorganic binder iSys MTX (CHT, Germany), plain plated knits made of pure cellulose fibres, cellulose and synthetic PA and PES fibres blends, and pure PES fibres demonstrated different antimicrobial activity against Gram-negative (*E. coli*) bacteria than that against Gram-positive (*S. aureus*) bacteria. It was determined that according to the qualitative standard (EN ISO 20645:2004) a good antimicrobial effect is provided only by antimicrobial treated pure cotton and man-made bamboo plain plated knits. However, evaluating the antimicrobial activity in blended knits using this method was rather complicated as opposite sides of the plated knit showed different antimicrobial effect. For pure polyester fibres the knit showed no antimicrobial effect.

A more accurate evaluation of the antimicrobial properties of both sides of plated knit was carried out using the modified quantitative Antibacterial Finishes on Textile Materials Test method. The pure cotton knits exhibited a substantially greater bacterial reduction (up to 86% against *E. coli* and up to 78% against *S. aureus*) than the other plated knits investigated, even more than man-made bamboo knits (their bacterial reduction was up to 70% against *E. coli* and up to 65% against *S. aureus*). The growth of bacteria on the polyamide knitted fabrics plated with cotton and man-made bamboo yarns was 11 - 18% less with *E. coli* bacteria and 13 - 25% less with *S. aureus* bacteria compared with the pure cellulose fibre knits. The difference in antimicrobial activity of PES knits plated with cotton and man-made bamboo yarns was 45% less against *E. coli* and 53% less against *S. aureus* bacteria compared to the pure cellulose fibre knits.

It was found that the adsorption of antimicrobial agents on the fibre surface is influenced by the fibre structure of knits, which is important because between a certain type of fibre and antimicrobial agent a bond may not occur, and that would have a negative impact on the antimicrobial activity.

The antimicrobial activity of plated knits also depends on the yarn construction. Antimicrobial treated knits with the same fibre composition and very similar total linear density of yarns but with a different number of folded yarns in the structure have different antimicrobial activity because the higher number of folded yarns in the knitted loop has a larger surface area and, herewith, larger antimicrobial acting area.

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