Chapter 9

Efficiency of Medical Workers' Uniforms with Antimicrobial Activity

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Additional information is available at the end of the chapter

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Abstract

Antimicrobial finishing of textiles protects users from pathogenic microorganisms, which can cause medical and hygienic problem. The use of such textiles particularly increases in healthcare facilities, where reduction and transmission of pathogenic bacteria are important factors for preventing nosocomial infections. In the present study, the efficiency of fabric with silane quaternary ammonium compounds (Si-QAC) applied as active agents was evaluated. A test was performed according to ATCC 100-1999 Test Method after 0-, 24- and 48-hour incubation times. The treated textiles were effective against *Enterococcus faecalis, Staphylococcus aureus* and *Klebsiella pneumoniae*, but were not effective for Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. Testing was also performed in hospital environment at infectious department where working clothes made of treated fabric were compared to normal working clothes. Antimicrobial textiles were not effective in a hospital environment, where average microbial count on medical workers' uniforms without antimicrobial protection was 1.4×10^9 cfu/mL, and 1.3×10^9 cfu/mL for uniforms made of antimicrobial material. Our conclusion is that quantities of application rates for Si-QAC should be higher or should be improved with applying another antimicrobial coating to obtain complex with dual activity.

Keywords: antimicrobial textiles, silane quaternary ammonium compounds, antimicrobial finished textiles test methods, medical workers' uniforms, healthcare-associated infections

1. Introduction

Hospital textiles, together with moisture and heat, create the right conditions for growth, proliferation and long-term survival of many microorganisms, which can serve as a vector of cross-transmission of healthcare-associated infections (HCAI) [1, 2]. Medical workers'

uniforms can easily become contaminated and there are several reports of uniforms contamination by pathogenic microorganisms [3–5]. HCAI do not only represent complications in the treatment of patients [6, 7], but also cause economic damage with annual financial losses in Europe estimated at 7 billion EUR. Therefore, the medical institutions are increasingly using fabrics with antimicrobial activity, for reducing the transmission of HCAI. Various antimicrobial textile materials are developed using a variety of active agents including triclosan, metals and their salts, phenols, quaternary ammonium compounds (QAC), and organometallics [8]. The aim of our study was to test the efficiency of fabric with silane quaternary ammonium compounds (Si-QAC) applied as active agents in laboratory environment as well as in hospital environment.

2. Properties of antimicrobial textiles

The finishing process in textile manufacturing industry gives special, enhanced antimicrobial characteristics to the fabric, that can be biocidal (i.e. include agents that kill microorganisms) or biostatic (i.e. inhibit the microorganisms' growth). Various antimicrobial textile materials are developed using a variety of active agents which include synthetic antimicrobial agents such as triclosan, metal and their salts, phenols, quaternary ammonium compounds (QAC), and organometallics. When evaluating antimicrobial treated textiles the principle of antimicrobial agent fixation (**Figure 1**) to the fabric is crucial, since it is closely related to the mechanism of antimicrobial activity [9, 10]. Leaching antimicrobials that are not chemically bound to the textile are gradually and persistently released from the textile into their surroundings where they demonstrate their antimicrobial activity. On the other hand, the chemically bound antimicrobials are bound to the surface of the textile fibres and act as a barrier for the microorganisms with which they come into contact (principle of bio-barrier formation) [11, 12]. Some of the most common antimicrobial textile finishes, their principles of functioning with positive and negative features are presented in **Table 1**.

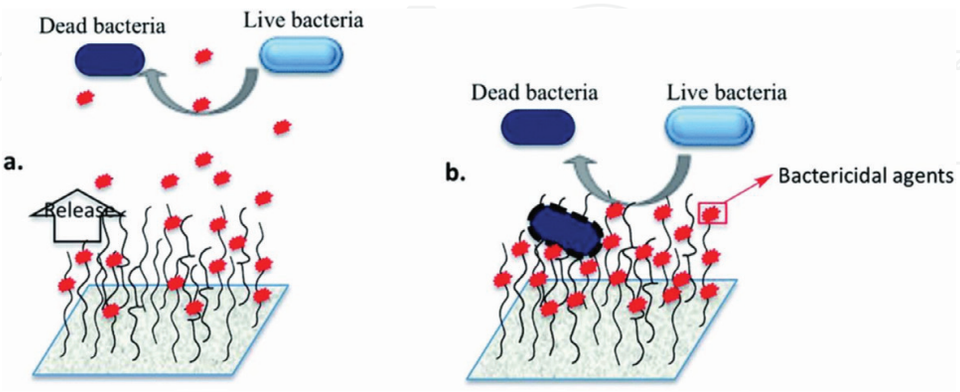

Figure 1. Principle of antimicrobial agent fixation and mode of action (a) leaching antimicrobial agent released from the textile, (b) contact killing coating bonded to the textile [11].

Antimicrobial agent	Principle of functioning	Positive/negative features
N-halamines [13, 14]	Electrophilic substitution of Cl in the N-Cl bond with H in the presence of water and results in the transfer of Cl+ ions that can bind to acceptor regions on microorganisms. This hinders enzymatic and metabolic processes, leading to the destruction of the microorganisms	+ biocides that are active for a broad spectrum of bacteria, fungi and viruses – as an N—H bond, which does not have antimicrobial properties, is formed in the substitution reaction, further exposure of the agent to dilute sodium hypochlorite is needed for regeneration of its antimicrobial activity
Triclosan	By using electro chemical mode of action active substance penetrate and disrupt cell wall, leading to leaking of metabolites and disabling cell functions [15]	+ it is not water solubel and does not leach out [16]
Chitosan [17–19]	Positively charged amino groups can bind to the negatively charged bacterial surface, resulting in the disruption of the cell membrane and an increase in its permeability. Such antimicrobial function is very similar to that determined for QAS. Chitosan can also interact with the DNA of microorganisms to prevent protein synthesis	+ nontoxicity, biocompatibility and biodegradability – weak adhesion to cellulose fibres, resulting in a gradual leaching from the fibre surface with repetitive washing
Metals (Ag, Co, Zn), metal compounds and nanoparticles of metals [20, 21]	Oxidative stress resulting in damage to the lipids, binding and inactivation of intracellular proteins [22], inhibiting active enzyme centres in microorganisms [16] and losses replication ability of microorganisms DNA	+ nano-sized inorganic particles possess high surface area/volume ratio and display unique physical and chemical properties Ag: low toxicity to animals' cell [23]
Bioactive Plant-based Antimicrobial Agents [24, 25]	mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters	+: enable the production of safe, nontoxic, skin and environment friendly bioactive textile products
QAC [26–30]	Interactions between the cationic ammonium group of the QAC and the negatively charged cell membrane of the microbe; these interactions consequently result in the formation of a surfactant–microbe complex. This in turn causes the interruption of all essential functions of the cell membrane: the denaturation of proteins and interruption of protein activity, causing disruption of the cell structure [22]	+: active against a broad spectrum of microorganisms such as Gram-positive and Gram-negative bacteria, fungi and certain types of viruses −: QASs have an inherent weakness: leaching from the textile. To fix a QAS on textile fibres, sol–gel technology has also been used in antimicrobial textiles. This enables the formation of a nanocomposite polymer network with an organic–inorganic hybrid structure (e.g Si-QAC)

Table 1. Principles of functioning with positive and negative features of selected antimicrobial textile finishes.

Today, there are different available antimicrobial agents on the market to give textiles antimicrobial characteristics. Each antimicrobial agent has different modes of action and is chosen according the end use of a textile product [31].

2.1. Methods for application of antimicrobial agents

Antimicrobial agent can be applied by different methods. Active agent can be added before the polymer extrusion in the spinning process producing fibres or films. In case of antimicrobial fibres, the antimicrobial agent is integrated into the fibre, while antimicrobial films act as a barrier to microorganisms, but can make fabric impermeable to airflow leading to heath stress [32, 33].

Another procedure to obtain textile with antimicrobial protection is adding the active agent in the phase of textile finishing, where active agent can be applied on fibres or on the flat surfaces. The application can be obtain during processes of exhaustion, pad-dry-curing, coating, spraying and foam technique [16, 32].

2.2. Functionalization of textiles by Si-QAC

Quaternary ammonium compounds (QAC) are a chemical class of cationic surface active agents [12]. Once the microorganisms are exposed to the QAC, the agent absorb and penetrate into the cell wall where it reacts with the cytoplasmic membrane (lipid or protein) leading to membrane disorganization. Low-weight intracellular material is leaking out, which leads to degradation of proteins and nucleic acids and finally autolytic enzymes cause lysis of the cell wall [34, 35]. Although being chemically bonded to the textile fibres and their concentration does not change with time, they cannot ensure the permanent antimicrobial activity of agents because the settling of dead microorganisms on the bio-barrier can greatly reduce or even eliminate their effectiveness [36]. There is proven antimicrobial activity of QAC on natural cellulosic fibres, polyester/cotton blends and secondary acetates [16, 22].

The active ingredient dimethyltetradecyl[3-(trimethoxysilyl)propyl]ammoniumchlorid of tested antimicrobial agent consist of a Silan group, positively charged ammonium group and long nonpolar chain (**Figure 2**). The antimicrobial agent is applied using Foulard process. In the clamping frame, the Silan group is bound to the fibre using heath and a condensation reaction, where QAC agent is covalently bound to the fibre (**Figure 3**) [37]. The permanent connection with the neighbouring molecules is formed and the active agent evenly surrounds the fibre.

The SEM imaging of fabric samples (**Figure 3**) shows that covalent bounding of active agent did not cause any noticeable degradation of the fibres and did not affect fibre morphology, indicating that important functional and aesthetic qualities of the fabric were retained. There are no obvious film layers and particles attachments on the treated textiles surfaces.

2.3. Durability of antimicrobial treatment using Si-QAC protection

Ideal antimicrobial agents should be effective against a broad spectrum of agents, have low toxicity, be compatible with other finishes, be easy to apply and also be durable and

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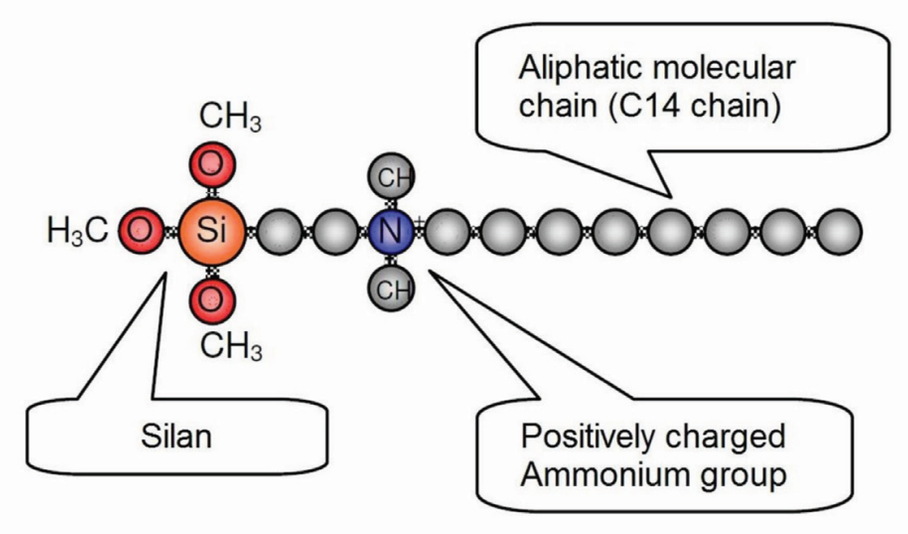

Figure 2. Schematic chemical structure of tested antimicrobial agent.

persistent in washing process. Therefore for many textile applications the wash durability is a key parameter for assessing the performance of antimicrobial treatments [31]. Durability is fundamental to ensuring the antimicrobial performance throughout the life cycle of a textile product. Washing and wear parameters, which can of course lead to a loss of the antimicrobial efficiency, are the key factor for assessing the antimicrobial performance. It is expected that a durable antimicrobial textile finish should survive at least 50 machine washes in line with industrial practices [38]. In our survey Si-QAC treated textiles were washed according to RAL-GZ 992 procedure in 25 and 50 cycles. Antimicrobial protection was decreasing with the number of washing cycles and was totally lost after 50 washing cycles.

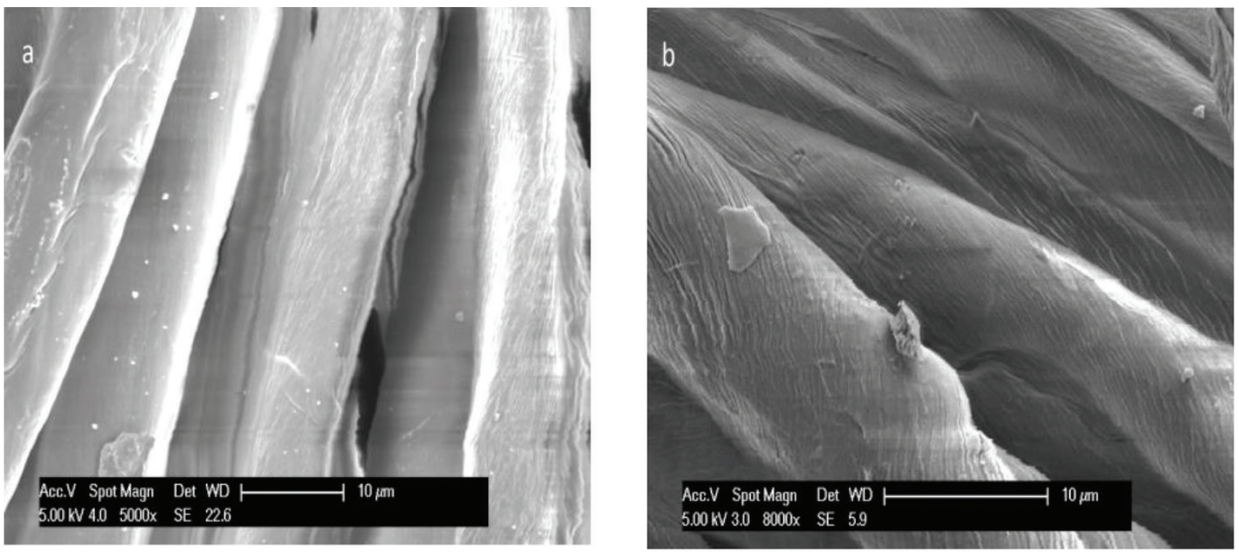

Figure 3. Micromorphology of the textile fibres. (a) SEM image of raw textiles. (b) SEM image of Si-QAC treated textiles. Magnification: 5000×.

3. Testing and characterization

3.1. Standards and test methods

Proof of activity against bacteria can be performed according to accepted standards and test methods where tested material (e.g. fabric) are compared to standard or an identical material without antimicrobial finishing.

3.1.1. JIS L 1902:2008 (testing for antibacterial activity and efficacy on textile products)

This standard specifies the testing method for evaluating the antibacterial activity against bacteria on antibacterial finished textile products. Using one of the following methods:

- Qualitative test (halo method) to evaluate antibacterial activity of textile products by the existence of halos, and applicable to those of which antibacterial finishes chemicals can diffuse into the plate agar culture medium.
- Quantitative test to evaluate antibacterial activity of textile products by bacteriostatic activity value, bactericidal activity value, bacterial decrease value. The absorption method shall be applied for high humidity conditions and printing method for low humidity conditions. Bacteria are shaken out after the incubation of inoculated test pieces.

The measurement of bacterial concentration shall be carried out by viable plate counting or luminescence method for determining ATP (Adenosine Tri-phosphate) concentration [39].

3.1.2. DIN EN ISO 20743

This International Standard specifies quantitative test methods to determine the antibacterial activity of all antibacterial textile products including nonwovens, with different types of used of antibacterial agents or the application methods. The user can select out of three inoculation methods for antibacterial activity determination:

- Absorption method where the test bacterial suspension is inoculated directly onto tested surfaces;
- Transfer method where the test bacteria are placed on an agar plate and transferred onto tested surfaces; and
- Printing method where the test bacteria are placed on a filter and printed onto tested surfaces.

For measuring the enumeration of bacteria colony, the viable plate counting method and the ATP luminescence method are also specified [40].

3.1.3. AATCC 147 (parallel streak method)

The objective of the method is detecting bacteriostatic activity on textile materials. The method is useful for obtaining a rough estimate of activity. Increasing degrees of sensitivity is indicated

where growth of the inoculum organism is decreasing from one to the other end of each streak and from one to the next streak. Tested surfaces are placed in intimate contact with the inoculated (with test bacterium) agar surface and incubated. Antibacterial activity of the tested material is indicated in clear area of interrupted growth underneath and along the side of the test material [41].

3.1.4. AATCC Test Method 100-1999 (antibacterial finishes on textile materials: assessment of)

This method provides a quantitative procedure for the evaluation of the antibacterial activity degree. Test and control swatches are inoculated with test organisms and incubated. By shaking in known amounts of neutralizing solution, the bacteria are eluted from the swatches. The number of eluted bacteria is determined and percentage reduction by the treated specimen is calculated [42].

3.2. Study in laboratory environment

Textiles pieces (50/50 PES/COT white fabric, 195 g/m^2) with and without Si-QAC dimethyltetradecyl[3-(trimethoxysilyl)propyl]ammoniumchlorid were sterilized, dried, artificially contaminated (**Figure 4**) and tested for antimicrobial activity according to ATCC 100-1999 Test Method after 0, 24 and 48 hour incubation times. Ready-made cultures of *Escherichia coli* (DSM 1562),

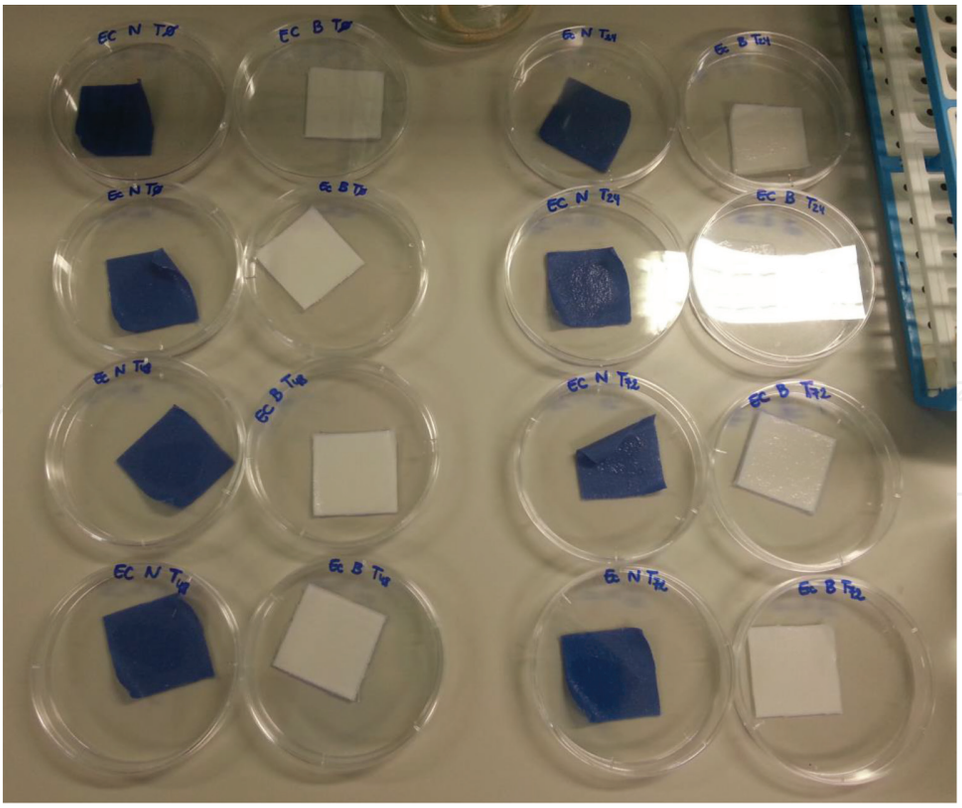

Figure 4. Artificially contaminated tested textile pieces with *E. coli* suspension (first and third column textile pieces with Si-QAC, second and fourth column textile pieces without Si-QAC).

Enterococcus faecalis (ATTC 29212), *Klebsiella pneumoniae* (ATCC 13883), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were taken from the freezer and grown in nutrient broth (Tryptiyc Soy broth) for 4 days in an incubator at 37°C. Efficiency rate after 24 or 48 hour incubation was calculated using serial dilution, viable plate counting and colony forming units per millilitre (cfu/mL) calculation. Appropriate selective growth medium (e.g. VRBD agar, Kanamycin Esculin Azide agar, *Klebsiella pneumoniae* selective agar, Baird Parker agar, Cetrimide agar) were used.

The performance of fabrics with antimicrobial activity varies and depended on tested microbial species and it is to some extent related to the bacteria Gram categorization (e.g. Gram negative and Gram positive). When comparing textile with antimicrobial activity to nontreated textile the differences in reduction rate were 3.22×10^2 cfu/mL for *E. faecalis*, 1.87×10^4 cfu/mL for *S. aureus* two representatives of Gram-positive bacteria and 6.81×10^3 cfu/mL for Gram-negative *K. pneumoniae* (**Figure 5**), but treated textiles was not effective for Gram-negative *E. coli* and *P. aeruginosa* (**Figure 6**).

QACs are membrane active agents with a target site predominantly at the cytoplasmic membrane [43]. Since the cell wall structure varies between G+ and G− bacteria, the differences in sensitivity to QAC are expected. The cell wall of G+ bacteria represents much less effective barrier for the entry of antiseptics and disinfectants, which may explain the sensitivity of these organisms to many antibacterial agents including QACs [44–46]. Gram-negative bacteria are generally more resistant to antiseptics since their outer membrane acts as a barrier that limits

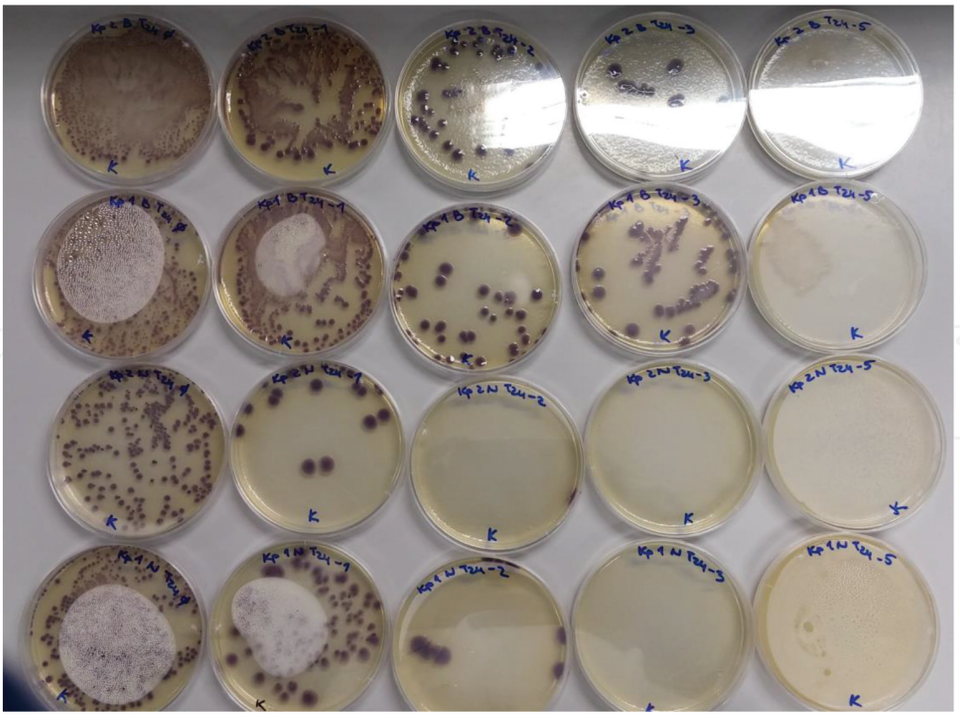

Figure 5. Colonies of *K. pneumoniae* after plating serial dilutions of suspension obtained from artificially contaminated textile pieces that were incubated for 24 hours. First and second row: samples without Si-QAC, third and fourth row: samples with Si-QAC.

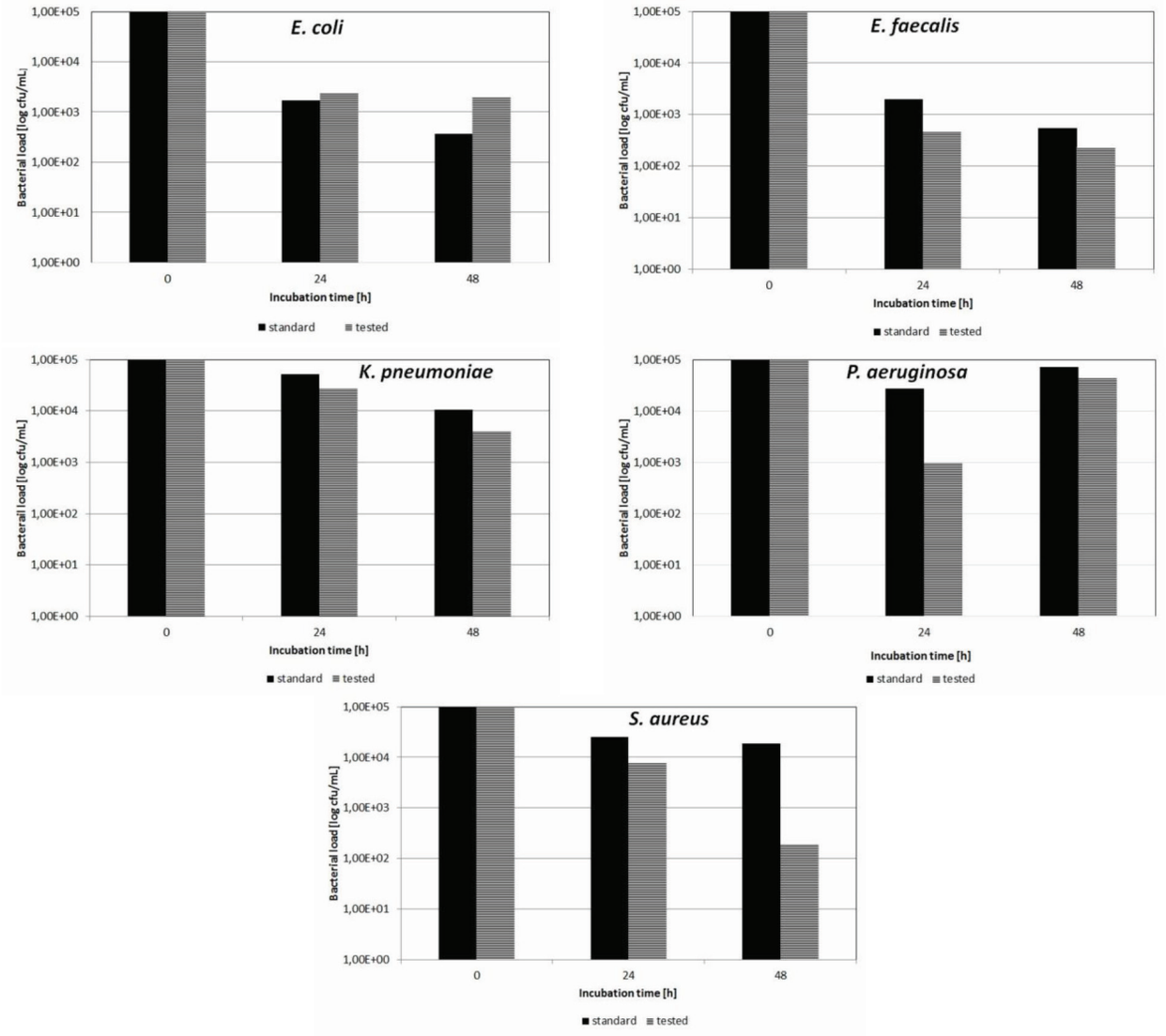

Figure 6. Bacteria per mL of eluting solution after 0-, 24- and 48-hour incubation time for 1×10^5 cfu/mL inoculum on standard and tested textile pieces.

the entry of many chemically unrelated types of antibacterial agents [47–52]. The formation of biofilm (in case of *P. aeruginosa*) can also account for the reduced sensitivity of bacteria [53].

3.3. Study in hospital environment

Medical workers' uniforms were made of antimicrobial material and worn for one day at infectious department by 10 nurses. Normal working clothes were used for negative control. Testing was repeated in two cycles. At the time of testing patient contaminates with MRSA (methicillin-resistant *Staphylococcus aureus*), CRE (carbapenem-resistant Enterobacteriaceae) and bacteria producing ESBL (extended-spectrum beta-lactamases) were hospitalized on the department. After eight-hour shift, uniforms were transferred to laboratory (each uniform in separate sterile bag) and tested for presence of microorganisms. Method of sterile elution was performed as follows: 2 L of sterile elution solution (0.9% NaCl + 0.2% Tween 80) (JIS L 1902,

2008) was added to uniform in a sterile bag and shaken for 30 min at 300 rpm on a shaking machine (Heidloph vibramax 100), after 10, 20 and 30 min uniform was gowned per hand in the closed bag. 100 mL of eluate was sterile filtrated through 0.8 μm 0.45 μm membrane filter which was further transferred to 100 mL 0.9% NaCl and shaken vigorously for 1 min. Efficiency rate after 24 or 48 hour incubation was calculated using serial dilution, viable plate counting and colony forming units per millilitre (cfu/mL) calculation. MALDI-TOF analysis of selected morphologically different colonies was used for microorganism identification.

Antimicrobial textiles were not effective in a hospital environment, where average microbial count on medical workers' uniforms without antimicrobial protection was 1.4×10^9 cfu/mL, and 1.3×10^9 cfu/mL for uniforms made of antimicrobial material. Species of *Acinetobacter lwoffii* (G−), Bacillus spp. (G+), *Pseudomonas aeruginosa* (G−), *Pseudomonas alcaliphila, Pseudomonas stutzeri, Pseudomonas manteili, Pseudomonas putida, Staphylococcus epidermidis* (G+), *Staphylococcus lugduensis, Staphylococcus warneri* and *Stenotrophomonas maltophilia* (G−) were identified. Our results are consistent with others who had tested uniforms with antimicrobial protection in the real life hospital environment. Burden et al. found no evidence that either antimicrobial scrub product decreased bacterial contamination of HCWs' uniforms or skin after an 8-hour workday [54] or experimental uniforms provided results only slightly in agreement with in vitro data [55]. Despite the fact that the effectiveness of an antimicrobial material was demonstrated in laboratory test, the effectiveness in hospital environment was not present. Bacterial load on medical workers' uniforms was much higher than the concentrations of microorganisms used in laboratory test method, which could also have an effect on the antimicrobial efficiency, since the adsorption of dead microorganisms can affect antimicrobial function of chemical bound agent [12]. Therefore, our conclusion is that quantities of application rates of Si-QAC should be higher. Another approach to improve the effectiveness of antimicrobial protection is the tailoring of different antimicrobial coatings to obtain complex with dual activity (e.g. applying silver chloride and Si-QAC composite) [36, 56–61].

4. Application potentials and environmental concerns

By implementing medical workers' uniforms with antimicrobial activity the risk for cross-transmission is reduced, while the fabric itself is protected against potential growth of newly microorganisms. Within the decrease of cross-infections, the protection of users (medical workers, patients, workers in food industry, etc…) is better; moreover, the comfort and well-being at workplace are improved.

4.1. Medical workers' uniforms, beddings, and patients' wear

Work clothing of health workers is uniform, has the agreed cut and colour, and is always clean and daily fresh [62]. Its purpose is to protect health workers from direct contact with the patient's secretions and consequently the transfer of microorganisms from patient to medical personnel and later to other patients [63]. The protective and preventing antimicrobial products can be used in different end application and they are ideal to be used in healthcare sectors. Workers' uniforms and bed sheets, which build microenvironment around human body, made of antimicrobial treated fabrics can for example protect humans against nosocomial

infections in the hospital environments. Antimicrobial treatment of textiles aimed for hospital environment is usually marketed with the function of preventing the growth of microorganisms [54]. Antimicrobials for textiles need to fulfil many different criteria including efficacy against microorganisms, suitability for textile processing, durability and a favourable safety and environmental profile [31]. In the field of medical textiles, Ag (silver) and Si-QAC agents are widely used [31].

4.2. Preventing cross-contamination and HCAI

The origin of infection can be an infected person (e.g. patients, health workers or visitors) the environment. Microorganisms can be part of the patient's normal flora that can cause the nosocomial infection during the process of diagnosis, treatment and care of immunocompromised patients. Microorganisms are able to survive on environmental surfaces up to several weeks [64] providing a significant biotransfer/cross-contamination/cross-infection potential [65] that should not be overlooked. Microorganisms can also survive in the patient's abiotic environment, such as contaminated equipment for care, diagnosis and treatment (textiles), food, water, and disinfectants and on surfaces [66], which shows that one of the possible sources of nosocomial pathogens can be inappropriately disinfected textiles [67]. The environment can play a marked role in the nosocomial transmission of microorganisms [68], where garments of healthcare workers are an important aspect of the environment that can easily become contaminated and are therefore recognized as a possible vehicle of nosocomial infections. Hospital textiles could be a source of HCAIs, contributing to the transmission of pathogens both through indirect contact, via hospital staff, endogenously, and by means of aerosols [68, 69]. The characteristics of the textile in question, together with humidity and heat, can create the right conditions for the proliferation of numerous microorganisms [2, 3]. Pathogenic bacteria such as MRSA [3], *P. aeruginosa* and *K. pneumoniae* [4] and *C. difficile* [5] were also detected on uniforms of physicians and nurses. Surveys show that hospital textiles can be the source of nosocomial infections with streptococci [70], enterococci [71], *Bacillus cereus* [72], staphylococci [73] and coliform bacteria [74]. In addition, there is continuously increasing trends of antimicrobial resistance among HCAI pathogens leading to raising healthcare costs, prolonged hospital stays, treatment failures, and sometimes death [75].

4.3. Impact on the environment and human health

Although synthetic antimicrobial agents effectively inhibit the growth of microbes, most of them are toxic, can cause adverse effects on human health, and have environmental issues [8]. Recommended application rate of a silane quaternary ammonium compounds are ca. 10,000 mg/kg [76]. Si-QAC consumption in textiles was 1128 tonnes in 2004, most of it being a particular type of Si-QAC [77]. Exposure pathways and potential health effects need to be considered in order to evaluate the safety of antimicrobial compounds for humans. Si-QAC is readily biodegradable (after 6 days, 70% of the substance is degraded) [78] and is also expected to rapidly hydrolyze [79]. The type of active material (free or bound), the concentration in the product, the routes of exposure and the frequency of use all influence the extent to which humans may be exposed to antimicrobials in a textile product [80]. Although Si-QAC is a corrosive chemical, the USEPA does not expect any severe effects of Si-QAC use on human health [79], there is however

evidence of skin sensitization for Si-QAC [78]. For any substance that addresses microorganisms there is a need to consider the potential for development of resistance. Although durable antimicrobials are increasingly popular for the consumer market, questions regarding their use will continue to rise regarding their effect on the evolution of resistant microbes [81]. Due to relatively large quantities of recommended application rates, the bacterial resistance should be considered. Most biocides used on commercial textiles can induce bacterial resistance to these substances, which can lead to increased resistance to certain antibiotics in clinical use [82], but any possible clinical significance of this remains to be tested [22]. Although the number of studies elucidating the association between antimicrobials resistance and resistance to clinical isolates has been limited, recent laboratory studies have confirmed the potential for such a link (e.g. transfer of triclosan resistance) in *E. coli* and *Salmonella enterica*. Thus, widespread use of antimicrobials may represent a potential public health risk in regard to development of concomitant resistance to clinically important antimicrobials [83].

5. Conclusion

Working cloths made of textiles with proven antimicrobial protection could be an ideal solution for areas with frequent infections such as healthcare facilities. Ideal antimicrobial agents should be effective against a broad spectrum of agents, have low toxicity, be compatible with other finishes, be easy to apply and be durable and persistent in washing process. Efficiency of antimicrobial textiles could be improved with higher application rates of Si-QAC or additional binding of another antimicrobial agent, but in this connection, the effect on antimicrobial resistance evolution should be considered.

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