COMPARATIVE SUSTAINABLE ANTIBACTERIAL TECHNOLOGIES FOR TEXTILE APPLICATIONS

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Abstract: The present study is both a review regarding the newest methodologies of application of antibacterial agents on textile supports, natural and chemical, and an antithesis focusing on the comparison between well known antibacterial agents, ranging from synthetic organic compounds such as thriclosan, chitosan, quaternary ammonium compounds, polybiguanides, N-halamines, to metals such as silver. Different methodologies of application, their evaluation, as well as their advantages are presented. Regarding the antimicrobial products, the review is dealing with antibiotics and antimicrobial peptides, on one side, and natural extracts as colorants, on the other side. The immobilization of known compounds from the peptabiotic class of peptides has been recently investigated through their covalent bonding to the cellulosic and previously grafted cellulosic substrates. The number of bio-functional textiles with antimicrobial activity has increased considerably in the last decades. Subsequently, technologies of the application of the antimicrobial agents need to adapt to the medical and hygienic textiles requirements, and not only. Antimicrobial textiles with nontoxic bioactive compounds used in the bio-medical field for the purpose of addressing hygiene in clinical and sensitive environments by minimizing the conditions for microbial colonization of textiles, are presented and investigated. Natural dyes are nontoxic, biodegradable and do not cause pollution and waste water issues, and may show antimicrobial properties. Antimicrobials based on naturally-derived products have been increasingly studied, such as green walnut shells, gallnut, citrus fruit, henna, pomegranate, raspberry extract dyes applied on cellulosic, proteinic and synthetic fibers supports. The review emphasizes the idea that alternative antimicrobial agents represent a suitable substitute for classic antibiotics and demonstrates potentially broad applications in medical textiles, being entitled as sustainable methodologies. The best antimicrobial technologies for textiles should minimize potential risks while providing durable functionality and associated benefits.

Keywords: antimicrobial textiles, safety protection, methods, peptides, natural dyes.

1. INNOVATIVE ANTIBACTERIAL TEXTILES

In the sustainable development of textile items the reduction of risks by prevention of the effects which are toxic and non-desirable to both human health and environment. Subsequently, the prevention of the infectious diseases is very relevant. Since humans want a clean environment, the antimicrobial medical textiles are widely used for the hygienic products, first aid items, products for the treatment and the prevention of various nosocomial infections, anti-allergic compresses for contact dermatites, advanced bandages, implantable devices [1,2]. The antimicrobial protective agents can be natural compounds and synthetic, bacteriostatic and fungistatic agents, since they can be enzymatic inhibitors [3]. Almost all the antimicrobial synthetic agents used on polymeric supports are biocides. To reduce adverse effects on the environment new materials based on biodegradable molecules are of great interest. In particular, textile manufacturers are looking at the production of fabrics made of natural fibers but opportunely functionalized for specific technical applications [4]. Among the many polymeric materials available, cellulose fibers are particularly attractive, being naturally occurring, and easy to functionalize [5,6,7]. In this regard, it is worth mentioning that cotton-based garments, functionalized with antimicrobial compounds, are already commercially available [1,4,8,9,10]. Protective and safe textiles are of fundamental importance for health care workers and for immuno-compromised or debilitated people who need to be protected from infections. Generally, the antimicrobial textiles currently on the market are made of synthetic polymers containing silver salts or silver nanoparticles [7] (for which toxicity on skin has not been completely made clear [11,12]. Basically, the active compounds are simply adsorbed or electrostatically linked to the cotton surface. Therefore, they have no washing fastness after a few cycles of washing [13]. Thus, a great demand for durable antimicrobial textiles based on nontoxic bioactive compounds is imperative.
To add new value to this topic, studies in the field of antibacterial peptides have been reported [14,15]. Their natural provenience induces their good tolerance by our body. Recently, a cysteine residue linked to a cellulose support showing antibacterial properties was reported [16]. The immobilization of a 9-mer peptide on cotton fibers provided the inhibition of the growth of bacterial strains [17]. Generally speaking, cellulose derivatization involves the hydroxyl groups of cotton on which esters and ethers (ethyl cellulose) can be prepared [18]. As responsive behavior, cotton can be used as a substrate for the solid phase’ synthesis of peptides (SPPS) and for combinatorial syntheses [19,20]. The appropriate method can be chosen according to the nature of the peptide to be bound to the cotton. Notably, step by step synthesis directly on the cotton allowed also the synthesis of Lys dendrimers, a useful expedient to increase peptide concentration on a tissue and, hopefully, antibacterial bioactivity. [21]. Chemoselectivity based method, is a promising alternative. However, this approach needs further improvements to increase the yield of peptide anchorage. The present study illustrates a few synthetic pathways and analytical procedures that should allow the precise design of peptide-decorated cotton tissues. In particular, the syntheses of antimicrobial textiles for medical environments are currently in progress. Antibacterial peptides produced by vertebrates, invertebrates, bacteria, and plants are important components of the innate immune system and have been considered as promising materials for novel antibiotics. The advantages of antibacterial peptides over conventional antibiotics are a broad antibacterial spectrum and unique antibacterial mechanisms [22]. A number of antibacterial peptides disrupt bacterial cell membranes through peptide membrane interactions. Resistance against membrane disruptive antibacterial peptides is very unlikely to occur rapidly in bacteria because the target is unlikely to change in a short time. However, antigenicity and stability are problems with antibacterial peptides in therapeutic applications. Truncation of a peptide chain and use of D-amino acids were tested to resolve these problems. A series of 9-mer peptides have antimicrobial activities against fungi, and Gram-positive and Gram-negative bacteria were designed and synthesized. In addition, D-enantiomers of these peptides showed higher activity against Pseudomonas aeruginosa than their original L-enantiomers. Silk sutures and silk fibroin films impregnated with these peptides also exhibited the abilities to suppress proliferation of MRSA in vitro and in vivo [23]. Moreover, peptide-based natural antimicrobial agents for the biofunctionalisation of textiles at nanoscale could not only find a sphere of influence in the wellness sector but the ambition is to use them as prophylaxis and therapy tools. Indeed, several recent works demonstrated that solid substrates, such as a polymer and metal, immobilized antibacterial cationic peptides by covalent bonds to prevent bacterial proliferation and biofilm formation and reduce bacterial adhesion onto solid surfaces with long-term stability [24, 25]. Such approaches using antibiotics or antibacterial agents such as silver ions, quaternary ammonium ions, and pyridinium salts on solid surfaces have also been attempted to create antibacterial materials in the fields of medical devices, nursing care, and food packaging [26,27]. Design and development of antibacterial fibers on which antimicrobial peptides (i.e., D-peptide A and D) were immobilized with molecular chains by using the SPOT synthesis technique. It was demonstrated that the processed fibers exhibited potent antibacterial activity against S. aureus, including MRSA, and this activity was maintained after repetitive washing and sterilization by autoclaving. These results suggest that the fibers are promising candidates for novel therapeutic apparatuses especially for blood cancer treatments. AMP-immobilized antimicrobial cotton fibers with high durability have been developed by conjugation of the AMPs via covalent bonding. Immobilization is a complicated procedure, however, and the fibers could suffer chemical damage from reagents during the processing. Therefore, by using AMP and less severe conditions, a novel technique to obtain fibers with an antimicrobial finish was developed.

1. SYNTHETIC AND NATURAL BIOACTIVE COMPOUNDS

Several antimicrobial agents have been tested in textiles: Quaternary ammonium compounds, silver, polyhexamethylene biguanides (PHMB) and triclosan even in an industrial scale. They have powerful bactericidal activity, as indicated by the MIC value, and also different application methods, effectiveness on fibres depending on chemical composition, and side-effects, as reported in Table 1. However, the majority have a reduce spectrum of microbial inhibition and may cause skin irritation, ecotoxicity low washing resistance.
Table 1. Synthetic and natural agents used for the treatment of various polymeric supports [1,29]

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Fibre</th>
<th>Toxicity</th>
<th>Fibre interaction and side-effects</th>
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<tr>
<td><strong>Synthetic antimicrobial agents</strong></td>
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<tr>
<td>Silver</td>
<td>Polyester, Polyamide, Wool, Regenerated</td>
<td>Little to nontoxic</td>
<td>Slow release; durable but Ag can be depleted</td>
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<td></td>
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<tr>
<td>QACs</td>
<td>Cotton, Polyester, Polyamide</td>
<td>Moderate to highly toxic</td>
<td>Covalent bonding; very durable; possible bacterial resistance</td>
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<tr>
<td>PHMB (Vantocil)</td>
<td>Wool</td>
<td>Moderate acute aquatic</td>
<td>Large amount needed; potential bacterial resistance</td>
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<tr>
<td>Triclosan</td>
<td>Polyester, Polyamide, Cellulose acetate, Acrylic fibre</td>
<td>Breaks down into toxic dioxin</td>
<td>Large amount needed; bacterial resistance</td>
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<tr>
<td>N-Halamine</td>
<td>Cotton, Polyester, Polyamide, Wool</td>
<td>Moderate to highly toxic</td>
<td>Needs regeneration; odour from residual chlorine</td>
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<tr>
<td><strong>Natural antimicrobial agents</strong></td>
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<tr>
<td>Chitosan</td>
<td>Cotton</td>
<td>Encapsulation</td>
<td>Efficiency against microorganisms</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>Natural dyes (nuts, pomegranate, henna, antimicrobial peptides)</td>
<td>Cotton, Wool, Silk</td>
<td>Mordanting with citric and tannic acids, Covalent immobilization technique</td>
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Note: 'a' – inefficient; 'b' – efficient.

There are several studies concerning the application of natural dyes and natural plant extracts onto polymeric supports [30,31,32]. Especially, plant extracts are the most valued. Yet, there are several major challenges regarding extraction, isolation of the bioactive compounds, application and durability. Nevertheless, due to their ecofriendly nature and non-toxic properties they are still promising candidates as antimicrobial agents for textiles.

2. FIBRES AND FINISHES WITH ACTIVE ANTIMICROBIAL POTENTIAL

The antimicrobial agents can be added to the polymer before extrusion (fiber chemistry) or by post – treated of the fiber or the fabric during finishing stages. Some commercial bioactive fibres with antimicrobial silver treatment and finishing products are: SeaCell® Active, a cellulose-base fibre; MicroFresh®, SoleFresh® and Guard-Yarn®, polyester or nylon yarns with AlphaSan®, a zirconium phosphate-based ceramic ion-exchange resin containing silver; Trevira Bioactive®: polyester fibre with silver incorporated prior to the extrusion process; SmartSilver®, wool fibres with silver added by typical exhausting dyeing methods and other finishing silver-based products like SmartSilver™, Silpure®, Sanitized®, AlphaSan® and Ultra-Fresh. Cotton fibres are also being commercialised under a pre-treatment with Reputex® (PHMB attached to cotton) and more recently, polyamide with PHMB is sold as Purista®. Moreover, polyamide and polyester fibres treated with Tinosan AM 100®,
cellulose acetate yarns named Silfresh®, Microban® textile and Irgaguard® and Irgacare® products, all contain triclosan as antimicrobial agent. More recently, natural resources have been used for the development of a composite fibre of chitosan and viscose with durable antimicrobial activity (Crabyon® [31].

Table 2. The inhibition quantified as the average area of hybrid made of Pomegranate extract – polymeric cellulosic and proteic supports against S. Aureus and E. Coli

<table>
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<tr>
<th>Substrate</th>
<th>Concentration (%)</th>
<th>The inhibition average area (mm)</th>
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<tr>
<td></td>
<td>S. aureus</td>
<td>E. coli</td>
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<tr>
<td>Cotton</td>
<td></td>
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<tr>
<td>5</td>
<td>7</td>
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<tr>
<td>15</td>
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<td>3</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Wool</td>
<td></td>
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<tr>
<td>5</td>
<td>6</td>
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<td>15</td>
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<tr>
<td>30</td>
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Anticancer activity. The anticancer activity of D-9-mer peptide immobilized on cotton fiber against cancer cell lines was demonstrated by measuring cell viability.

Alternative methodologies with amino acids and antimicrobial peptides

In order to compare risks, and side effects associated with the use of synthetic antimicrobial agents along with the mild activity and durability associated with the above described natural compounds, new strategies polarized to natural defensive amino acids and peptides that are found in every living organism as new biocides for polymeric supports functionalisation [29,35,36]. AMPs fall into 4 principal categories based on their size, conformational structure, or predominant amino acid structure [35]. Virtually, all life forms express cationic AMPs as an important component of their innate immune defenses. The cationic peptides represent the majority of antimicrobial peptides already registered. Anionic peptides are a group of much smaller dimensions, only recently identified mainly in mammals. These peptides are small, hydrophilic and contain specific regions that confer a negative charge [37]. These cationic and anionic peptides exhibit broad-spectrum activity against Gram-positive and Gram-negative bacteria, yeasts, fungi and enveloped viruses [38,39,40].

2. METHODOLOGIES OF SUSTAINABLE FUNCTIONALISATION OF POLYMERIC SUBSTRATES

Biofunctionalisation of wool and polyamide fibres with L-Cysteine (L-Cys) was reported. A durable antimicrobial effect over Staphylococcus aureus and Klebsiella pneumoniae was obtained without cytotoxicity. In addition, due to the widespread resistance of bacteria to the available drugs, naturally occurring antimicrobial peptides (AMPs) are considered promising candidates for future therapeutic use. Furthermore, the peptides should have a broad spectrum against Gram-positive bacteria, Gram-negative bacteria and fungi. Few studies have exploited the immobilization of AMPs in several films through a covalent attachment. Bagheri et al. concluded that among the various methods of immobilization of AMPs, covalent attachment of two highly active α-helical peptides (that rendered the different substrates (resins) antimicrobial properties) offers several advantages, including long-term stability and lower toxicity of the AMPs. Generally speaking, cationic AMPs have the best antimicrobial activity. Hence, AMPs which can both be attached to polymers or form films are expected to bind several polymer-based textiles by exhaustion. To authors’ knowledge, this is a new strategy and approach aiming to mimetize nature through nanobiotechnological tools to give “protective skin” to polymeric matrices. Peptides generally contain a large number of functional groups that are available as binding sites for specific molecules. Such characteristics of peptides allow chemical or physical binding on surface-modified solid supports. Uncontrolled immobilizations often lose their biological activities because of sterific hindrance that inhibits peptide target interactions or correct folding of the peptide. To solve these problems, various peptide immobilization techniques via molecular chains were reported by early workers.
Different types of cotton functionalization

**Cotton Functionalization with Epibromohydrin and 1,2-Diaminoethane (C1)**

**Methodology:** Incubation of a cotton piece HClO₄ solution in DMF and epibromohydrin for 4 h; Washing of the with DMF and MeOH; Air-drying; Immersion in a solution of Fmoc–NH–CH₂CH₂–NH₂ and triethylamine in DMF for 12 h, and treatment of the functionalized cotton sample for 2 h with a solution of Ac₂O in DMF in the presence of triethylamine.

**Biofunctionalization of wool fibres**

A new biotechnological process using L-Cysteine (L-Cys) which provides a permanent, non-toxic and effective antimicrobial effect over wool-based materials was reported. The antimicrobial activity of the bioactive wool was assessed by the international standard JIS 1902:2002 against *Staphylococcus aureus* and *Klebsiella pneumoniae*, and the confirmation of L-Cys immobilization on wool substrates was assessed by the Ellman’s reagent (5,5'-Dithio-bis-(2-nitrobenzoic acid) (DTNB method).

**Nanobiofunctionalisation of wool**

Functionalisation of the wool material was performed by an exhaustion methodology: incubation of wool fabrics for 50 minutes at 60 °C, in order to preserve both the quality of wool and to ensure the reduced form of L-Cys aiming to endorse an increase in the number of free sulphhydryl groups on wool to achieve the desired antimicrobial properties; Rinsing; and Soaping procedure at 40°C, in accordance with the recommendations of the Standard NP EN ISO 105-C06, in order to give evidence of a durable biofunctionalisation effect.

**Functionalization of cellulosic and proteic substrates with natural colorant extracts**

Fabrics samples were treated with natural dyes, by using a method of post-mordanting with aluminium sulphate, as mordant. The substrates were treated with different concentrations of dyes, then rinsed with deionized water and dried in air.

**Alternative environment-friendly methodologies for polymeric supports finishing**

The finished antimicrobial polymeric supports have hygienic and medical applications, especially for neuro-dermatites and diabetic patients, by reducing the secondary infections occurrence. The antimicrobial effect can be provided by fixation onto polymeric supports of zinc, copper and silver ions, by sol-gel technique. The antimicrobial finishing without nano-particles is possible and can be achieved by loading of some clay based matrices with silver ions and their fixation by sol-gel technique [41]. Recent researches stressed that natural dyeing and anti-bacterial finishing of cotton supports have been efficiently performed by using a bio-waste – chitosan as. The values gathered by colour measurement, as well as antimicrobial features are comparable with those obtained by using the classic mordanting procedure with alum [42]. In addition, the antibacterial activity was visibly improved against *S. aureus* and *E. coli*, after dyeing. In order to extend the dynamic attributes of textile matrices, a bioactive material can be prepared by using inclusion compounds, having as physical adsorption support MCT–β–CD (mono-chloro-tryazinyl–beta-ciclodextrine). Within the CD inclusion compound some anti-alergic agents like, menthol with Viola tricoloris Herba, with anti-inflammatory and anti-alergic action, for dermatologic changes whose biologic activity and biocompatibility has been tested in vivo [43].

3. CHARACTERIZATION METHODS OF THE CONJUGATES, RESULTS, COMMENTS

The chemical changes occurred onto textile fibres surface as a result of peptides functionalization can be highlighted by Uv-Vis and FT-IR spectroscopy. The most representative absorption bands are as follows:

In case of referring to ligno-cellulosic support-β-ciclodextrine –extract of *amarena cherries composite*, the wavelengths values and their assignements to β-CD, natural colorant extract and β-CD/colorant natural inclusion complex onto linen supports can be explained due to the same vibrational modes (Fig.1).
The use of FT-IR technique permits us to detect the complex formations in solid phase and to show the involvement of the different functional groups of guest and host molecules in the inclusion complex formation by studying the significant changes in the shape and position of the absorbance bands of natural dye, β-CD, and inclusion complexes. The β-CD exhibited significant FTIR peak at wavenumber of 880.31, 1047.1515, 1398, 1637 cm$^{-1}$.

**Morphology characterization by scanning electron microscopy (SEM)**

To investigate the morphological changes caused by the chemical modifications, SEM observation was performed. Figure 2 shows SEM images of untreated cotton fiber and D-9-mer Peptide-Immobilized Fibers. These images demonstrate that the convolution and the spiral secondary wall which were characterized as cotton fiber were maintained after the chemical modifications.

In case of linen samples grafted with mono-chloro-triazinyl-cyclodextrine, SEM morphology provided information concerning adsorption mechanisms of black currant extract onto linen fibres. From Figure 3 it was clear that the interfibre capillaries were responsible for retaining a significant amount of pigment. Subsequently, uptake by the interfibre capillaries was the main adsorption mechanism rather than adsorption on the fibre surface. Linen fibre was observed to have several key properties such as hydrophobicity, good affinity for black currant extract, rapid adsorption on contact, and high adsorption and retention through interfibre capillaries.
4. TESTING OF ANTIMICROBIAL ACTIVITY

There are several standard methods to assess antimicrobial activity on textile materials because there is not a unique test that is suitable for all the sorts of the antibacterial fibres and microorganisms. These standards are classified on the basis of the kind of the evaluation of microorganism inhibition: qualitative and quantitative. Qualitative methods include AATCC 147:2004, ISO 20645:2004, SN 195920:1992, and JIS L 1902:2002 – Halo method for antibacterial assessment, and AATCC 30:2004, SN 195921:1992 and JIS Z 2911-1992 for antifungal assessment. Quantitative methods include AATCC 100:1999, JIS L 1902:2002– Absorption method and ISO 20743:2007 [34]. The most useful romanian standards are EN ISO 20645:2004 Textile fabrics-Determination of antibacterial activity- Agar diffusion plate test and EN 14119-2003- Testing of textiles-Evaluation of the action of microfungi. The results are expressed as the width of the inhibition zone( mm). These qualitative methods evaluate the bacterial activity by the halo formation (absence of bacteria growth around the edges of the test specimen). They also provide a formula to measure the inhibition zone width even though it cannot be considered as a quantitative indication of the antibacterial activity because the colonies are not counted. In addition, the halo size only provides some indication of antimicrobial activity against the tested strains. In contrary, quantitative methods provide values of antimicrobial activity based on the reduction of microorganism population [2,14] e.g. based on the number of bacteria still living after incubation with the bioactive specimen. However, they are more time consuming than the qualitative methods and a greater amount of test specimens is required [1,34.] e.g. These methods are of utmost importance to assess antimicrobial properties of textiles but strongly depend on the mechanism of action of the antibacterial agent, with or without migration effect, and on the hydrophobic/ hydrophilic character of the bioactive material. The reports stressed the antibacterial activity of plant extract against *Staphylococcus aureus* and *Escherichia coli*. The extract from pomegranate indicates the occurrence of antibacterial activity against Gramm-positive and Gramm-negative bacteria.

5 CONCLUSIVE REMARKS

The present report describes novel methods to give antibacterial performance to wool fibres using a non-toxic and biodegradable agent - L-Cysteine, a natural compound never studied before as a potential bioactive agent for textiles which can grant antibacterial properties without cytotoxicity. The major advantages of this method in comparison with others that are commonly used are the non-toxicity both to the potential users and to the environment, the high bioavailability once immobilized on wool, and the durability. In addition, because L-Cysteine is part of several living organisms it is not expected to cause bacterial resistance. This is totally new and open promising perspectives for the functionalisation of polymeric materials with AMPs which can have an effective antimicrobial activity against a broad spectrum of microorganisms. The presented antibacterial methodologies will be able to open new viables perseevctives for the medical area, and extended for other applications of textiles both in the sanitary and technical fields.
ACKNOWLEDGEMENTS

This work was supported by a Joint Research Project, of the Romanian National Authority for Scientific Research (ANCS) and Italian Ministry of Foreign Affairs, DG for Country Promotion, 2012.

REFERENCES

STUDIU COMPARATIV PRIVIND METODOLOGII ANTIBACTERIENE SUSTENABILE CU POTENȚIAL APLICATIV ÎN DOMENIUL TEXTILELOR

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Rezumat: Lucrarea se dorește a fi o sinteză privind cele mai noi metodologii de aplicare a agentilor antibacterieni pe suporturi textile naturale și chimice, dar și o antiteză centrandu-se pe compararea cu agentii antibacterieni consacrați începând cu compușii organici sintetici, precum triclosan, chitosan, săruri cuaternare de amoniu, polibiguanide, N-alamine, pâna la metale - ionii de argint. Sunt abordate metode diferite de aplicare a unor posibili agenti antimicrobieni, de investigare a materialelor tratate, dar și beneficiile aplicării acestor tratamente. Ca structuri antimicrobiene, sintezele construiște în atenție antibioticele și peptidele antimicrobieni, pe de o parte, dar și extractele de coloranți naturali, pe de altă parte. Metode de imobilizare a unor cunoscute peptabiotice din clasa peptidelor au fost recent investigate și au ca scop ancorarea lor stabilă, prin legături covalente de suporturile celulozice sau celulozice modificate. În ultimul timp textilele biofuncționale cu activitate antimicrobială au cunoscut o dezvoltare fără precedent. Tehnologiile de aplicarea a agentilor antimicrobieni prin intermediul ciclodextrinelor grefate pe suporturile textile sunt actuale și cuprind compuși gazdă, precum: acid ferulic, cafeic, etil ferulate, alantoina, etc. Ulterior se pot obține și efecte antialergice ce completează protectia antibacteriană prin utilizarea unor compuși naturali gen propolis cu Viola tricoloris Herba, mentol, etc. Studiul evidențiază ideea că, agentii antimicrobieni reprezintă o alternativă la folosirea antibioticelor standard și demonstrează potențialul lor de aplicabilitate în domeniul textilelor medicale, fiindu-le pe deplin recunoscute atributul de agenti bioactivi/biodisponibili sustenabili. Se impune deci ca cele mai bune tehnologii antimicrobiene aplicate în vederea obținerii textilelor medicale să minimizeze potențialele riscuri, în aceeași măsură în care le conferă o funcționalitate durabilă.