Chapter

Antimicrobial Agents for Textiles: Types, Mechanisms and Analysis Standards

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Abstract

The large surface area, and ability to retain moisture of textile structures enable microorganisms' growth, which causes a range of undesirable effects, not only on the textile itself, but also on the user. Moreover, textiles used in health care environments are required to possess antimicrobial property to minimize spread of pathogenic infection. Anti-microbial property can be imparted via chemical finishing with an antimicrobial agent. Currently the use of antimicrobial agents includes metal compounds (notably copper and silver particle), chitosan, halogenated phenols "triclosan", quaternary ammonium compounds, antibiotics (a class of antimicrobials produced from microorganisms that act against one another), and N-halamines. The possibility of bacterial resistance limits antibiotic use to specific medical applications, and triclosan is known for being dangerous to the environment and is currently under scrutiny for possible endocrine disrupting to human being. Although quaternary ammonium compounds are stable and easily manufactured, microbial resistance is also a concern. Quaternary ammonium compounds (QACs), Polyhexamethylene Biguanide (PHMB), chitosan and N-halamines are listed under bound or non-leaching type antimicrobials. The bulk of current chapter focuses on the different family of antimicrobial agents used for textiles and their mechanisms.

Keywords: Finishing textiles, nanoparticles, silver particles, quaternary ammonium, antibacterial effect, ecological antibacterial

1. Introduction

Microorganisms play both beneficial and harmful roles in our lives. Some of the beneficial roles include production of oxygen via photosynthesis, nitrogen fixation, circulation of carbon by decomposition of dead organic matter, formation of crude oil, and helping animals such as cows digest their food. They are used by humans in making bread, beer, cheese, and antibiotics. Some of the harmful effects are caused by the virulence of pathogenic microorganisms, i.e., infection causing bacteria such as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *Enterococcus faecalis* (*E. faecalis*). Health care associated infections can be controlled by inhibiting the

various routes of transmission that causes an infection to spread from an infected person to healthy person. One of the various routes through which an infection can spread is the direct contact with infected individuals; infected water and food; contact with inanimate objects such as textiles used in scrubs, doctor's coats, surgical gowns, bed-sheets, pillow covers, and curtains. The control of the spread of infections via infected individuals, water and food can be achieved by developing hygienic practices.

Textiles have been recognized as a media for the growth of microorganisms such as fungi and bacteria. The growth of these microorganisms on textiles inflicts unwanted effects not only on the textile material, but also on the consumer. These effects can include the generation of unwanted odor, discoloration in the fabric, an increased probability of contamination, and an overall reduction in the fabric mechanical strength [1, 2]. The spread of infections through textile materials can be controlled by the use of antimicrobial textiles that kill pathogens on contact or hinder their ability to reproduce prior to being transferred on to another material or person. Antimicrobial textiles are made by treating textile substrates with antimicrobial agents or by using textile fiber with inherent antimicrobial efficiency. Antimicrobial agents are bound to textiles by different methods depending on the chemistry between the antimicrobial agent and the textile [3]. Consumers' attitudes toward hygiene and their desire for comfort and well-being have created a rapidly increasing market for antibacterial materials. Therefore, there has been extensive research in recent years in this area. Estimations have shown that there was approximately a production of 30,000 tons of antimicrobial textiles in Western Europe and 100,000 tones worldwide in year 2000. It was also estimated that the production increased by over 15% annually in Western Europe between 2001 and 2005, making antimicrobial textiles a rapidly growing sector of the textile market [1]. While synthetic fibers have been known to be more resistant to microorganisms due to hydrophobicity, natural fibers are more vulnerable to microorganism attack. In addition, soil, sweat, and dust can be nutrient sources for microorganisms [2]. Socks, active-wear, shoe linings, and lingerie account for approximately 85% of the total antimicrobial textile production. In addition, there has recently been a large market for antimicrobial fibers in air filters, outdoor textiles, upholstery, and medical textiles [1].

Other than the antimicrobial ability, there are certain basic requirements to be satisfied by an antimicrobial agent for its successful application on textiles rendering them to be used commercially. The basic requirements of a good antimicrobial agent for textile substrates are summarized below [3, 4]:

- Should possess affinity for specific fabric and fiber types.
- Be easy to apply on textile substrates.
- Be able to inactivate undesirable microbes while simultaneously not affect desired microbes.
- Inert to chemicals to which the textile might be exposed during processing.
- Durable to repeated laundering, dry cleaning, ironing and prolonged storage including resistance to detergents used to care for the textiles.
- Stable during usage without degrading into hazardous secondary products.
- Not adversely affect the user or the environment.

2. Antibacterial family

Many antibacterial product and chemistry are available in the current market using different technologies. Most antibacterial agents applied on textiles have been used for many years in food preservatives, disinfectants, wound dressings, and pool sanitizers. The attachment of these compounds to textile surfaces or their binding with the fiber can reduce their activity largely and limits the antibacterial agents' availability. In addition, the antibacterial agent can gradually be lost during the washing and use of the textile material. The most widely used antimicrobial agents for textile applications are based on metal salts (for e.g., silver), quaternary ammonium compounds (QAC), halogenated phenols (for e.g., triclosan), polybiguanide (for e.g., PHMB), chitosan, and N-halamines [5]. The aim of this section is to present the general family of antibacterial textile finishing.

In general, antibacterial agents can either kill the microorganisms (−cidal) or inhibit their growth (−static). Almost all the commercial antimicrobial agents used in textiles (silver, polyhexamethylene biguanide (PHMB), quaternary ammonium compounds, and triclosan) are biocides. They can damage the cell wall or disrupt the cell membrane permeability, and inhibit the activity of enzymes or synthesis of lipids, while all these functions are essential for microorganism's survival [3].

The antibacterial material can be separated in two categories: antimicrobials with controlled release or 'leaching' mechanism and bound or non-leaching type antimicrobials. The mechanism of the leaching type will act upon contact of the cell. On the other hand, the non-leaching types will diffuse a disruptive chemical to the cell. This type is preferred for an environment supporting the diffusion of the chemical, such as water.

2.1 Antimicrobials with controlled release or 'leaching' mechanism

The antimicrobial agents that belong to this category do not form strong bonds with the textile substrate. The chemical species responsible for biocidal activity are released slowly from the treated fabric surface, thus killing all the microbes surrounding the agent. An advantage of leaching antimicrobials effect are their superior antimicrobial activity than compounds based on other modes of action on the same fabric under similar environmental conditions [6]. The flip side is that the antimicrobial agent in the textile substrate is depleted eventually and loses its effectiveness. Metal salts (e.g., silver) and halogenated phenols (e.g., triclosans) are examples of antimicrobial agents that utilize the leaching mechanism [7].

2.1.1 Metal and metal salts

The interest of metal and metal oxide particle reside in the high antibacterial activity against microorganism, durability and stability, while having a low mammalian cell toxicity, meaning they are safe for close to the skin application. Even at very low concentrations, many heavy metals are toxic to microorganisms. Metal particles are synthesized from different precursor and reducing agent to obtain different end material, morphology or to lower the impact of on cost or environment. Plethora of synthesis reaction are available from the scientific literature. However, the reaction principle is similar for each technique, using a sol–gel. The precursor is usually a water-soluble salt such as silver nitrate, copper chloride, and zinc acetate. The metal ion is reacted with a reducing agent, such as conventional reducer like sodium borohydride, citric acid, citrate, and ascorbic acid, or with bio-based reducer such as glucose, polysaccharide, cellulosic fiber and plant or microorganism

extract. The precursor is mixed with a reducing agent under different conditions such as heat, mixing, sonication to surpass the activation energy of the reaction. Strong reducing agent will require milder reaction condition, while weak reducing agent will require stronger reaction condition. During the reaction, the particle could be stabilized using a capping agent in order to control the shape, size and stability of the final product. In some reaction, the reducing agent will also be used as a capping agent. While metals such as zinc, cobalt and copper have had some applications in past years as antibacterial agents for textiles, silver, having an MIC value of 0.05–0.1 mg/L against *E. coli* is still the most widely used metal in textile applications and wound dressings [3, 7–9]. Moreover, this metal is less toxic in the human body than other heavy elements with a smaller risk for exposure through inhalation, ingestion, or dermatological exposure [10]. It was found that AgNP was much less toxic to human cells than silver ion [11]. A concentration of silver ion higher than 1 μg/ml is toxic to human mesenchymal stem cells while the concentration of AgNP can be higher than 2.5 μg/mL. AgNP can destroy bacteria even at a nano-molar level while silver ion needs to be at a micro-molar level.

Silver can be applied in other forms: silver ion exchangers, silver salts, and silver metals. Silver zirconium phosphate and silver zeolites are examples of ion exchangers. Silver chloride (AgCl), nanosilver chloride, and AgCl microcomposites (AgCl nanoparticles attached to titanium dioxide as a carrier material) are types of silver salts. Silver metal can be used in the form of filaments and silver metal composites [12]. With concerns regarding bacterial resistance to silver [3], there is efforts to increase the efficiency of metal-based antimicrobial. Other metal based antimicrobial agents found to exhibit good antimicrobial properties are based on copper and zinc compounds, in the form of their sulfides and sulfates [13]. Many studies on metal salts have focused on preparation of nano sized metal particles, which has led to the development of new generation of biocides [5]. Above all, AgNP (Silver Nanoparticles), a nanometric form of silver element without an ionic charge, can be used as a catalyst, an optical sensor and an antibacterial agent [14–16]. The antibacterial activities of the silver ion and salts are well studied, but research about antibacterial mechanism of AgNP is relatively recent [14]. Different methods have been developed to synthesize and incorporate AgNP in some biomedical applications, and some reports have proven AgNP to be a potent antibacterial agent, that is effective against both Gram-positive and Gram-negative bacteria [17–19].

2.1.1.1 Mechanisms of metal and metal salts antimicrobial action

All silver-based antimicrobials generate and release different amounts of silver ions, with silver metals releasing the least, silver ion exchangers releasing the most, and silver salts somewhere in between [20]. In the presence of moisture, silver releases ions that bind the bacterial cell's surface with proteins. On binding, the following action occurs [21].

- Denaturing effect of the silver causes DNA to get condensed and lose its replication abilities.

- Induces inactivation of bacterial proteins by reacting with thiol group [21, 61].

The form of the silver used impacts its antibacterial effectiveness. For example, a concentration of $AgNO_3$ should be higher than 1 mM to kill silver resistant E.coli. While only 80 nM of AgNP is necessary for the same result [17]. The antibacterial efficacy of silver is directly proportional to the amount of bioactive silver ions released in the presence of moisture, as well as its ability to penetrate bacterial cell

membranes [10]. Silver is effective at low concentrations and promotes wound healing without appreciable toxic risk. However, there is a small risk of developing allergies to silver [22, 23]. In fact, silver and copper ions can disrupt or kill the microbes via different mechanism path. First, the ions can diffuse trough the cell membrane and bond to the enzyme of the cell. The enzymatic activity of the cell is decreased, which inhibit the growth of the cell until the death of the cell. Second, Silver ion can kill microbes by binding to intracellular proteins and inactivating them, can inhibit the synthesis of ATP (Adenosine triphosphate) and lead to DNA (Deoxyribonucleic acid) denaturation [24]. To observe the killing mechanism of silver ion more directly, TEM (Transmission electron microscopy) and X-ray techniques were used to facilitate the investigation. When *Escherichia coli* (E.coli) and *Staphylococcus aureus* (*S. aureus*) were treated with $AgNO_3$, the cytoplasm membrane detached from the cell wall. Subsequently, DNA and protein failed to function and finally the cell wall was damaged [21]. Third, the silver cation can damage bacterial cell walls, proton leakage and result in cellular structural changes. It can induce proton leakage through the membrane of the cell and cell death. The silver cation is highly reactive in a concentration between 5 and 40 mg/L [25]. Regarding the AgNP, their exact antibacterial mechanism has not been clearly revealed to date [11, 26]. The reduced nano-silver did not show antibacterial activity toward E.coli, but when it was mixed with partially oxidized nano-silver, the mixture showed significant inhibition to the growth of E.coli. Thus, the antibacterial activity of AgNP is a result of surface oxidization as AgNP is sensitive to oxygen [17].

Others metals oxides of interest are titanium dioxide (TiO_2) and zinc oxide (ZnO). The mechanism of those compounds is believed to be mostly from the generation of reactive oxygen species (ROS). Those compounds prevent the antioxidant defense system and damage the cell membrane of the microbe. This mechanism is catalyzed by ultraviolet light. It is of particular interest as an adjuvant to the UV disinfection, which is of growing usage for disinfection against COVID-19. However, this also means the efficiency of those metal oxides is largely influenced by the environment in which they are used. The efficiency of the metal oxide could be reduced in the presence of antioxidant or pigment, often used in synthetic textile. The morphology of the particle will have a great impact on the stability of the product as well as the antibacterial activity. In general, the greater surface area will provide a greater activity, but decrease the durability for the leaching type.

Currently, silver is used in a large number of antimicrobial commercial textile products at a relatively low cost. The silver is in the form of ultra-fine metallic particles and is mainly applied to polyesters, in the finishing stage. Ruco-BAC®, SilverClear®, UltraFresh®, Silpure®, AlphaSan®, Microfresh®, Solefresh®, GuardYarn®, and SmartSilver® are some of the commercially available antibacterial agents applied on textiles [3, 27, 28]. In the case of synthetic fibers, metal and metal salts particles can be incorporated into the polymer prior to extrusion (or before electrospinning for nanofibers). For example, silver can diffuse into the fiber surface and in the presence of moisture it can form silver ions. Gradual release can lead to an extended period of antibacterial activity. In addition, silver nanoparticles can be padded onto cellulosic and synthetic fabrics, resulting in a durable antibacterial finish [29]. While metals and metal salts has excellent antimicrobial activity, leaching from treated textiles into laundering effluent is problematic. Ionic silver is highly toxic to aquatic organisms, with the EPA setting water quality criteria at 1.9 ppb in salt water and 3.4 ppb in fresh [30]. Effluent from both home laundering and industrial application can transfer silver into sewage treatment facilities, depleting necessary bacterial communities. Research conducted by Geranio et al. found that fabric treated with AgCl released only 2.7 ppb (2.4 ppb for AgCl plus a binder) of total silver per gram of textile after the first wash cycle [31]. As the

effectiveness of silver depends on the release of silver ions, too few ions result in a lack of antimicrobial action, and too many yield an excess leading to pollution and waste. Success depends on finding the balance between minimum antimicrobial concentration and effectiveness.

2.1.2 Halogenated phenols (Triclosan)

2,4,4′-trichloro-2′-hydroxydiphenyl ether, commonly known as Triclosan is a broad-spectrum antibacterial agent, having a Minimum Inhibitory Concentration (MIC) of less than 10 ppm against most kinds of bacterial species. Triclosan has been used since 1960 in a wide variety of consumer products including toothpastes, hand soaps, deodorants, mouthwashes, shower gels, etc. Its mode of action is inhibiting bacterial growth by blocking biosynthesis of lipids. As a relatively small molecule, triclosan can be used by exhaustion, combined with dyes, or applied after dyeing. Through melt-mixing or suspension polymerization, triclosan can be incorporated directly into synthetic polymers (**Figure 1**) [5, 32].

Triclosan inhibits the growth of microbes by using an electrochemical mode of action to penetrate and disrupt the cell wall of microbes. When incorporated within a polymer, it migrates to the surface and protects the material [3, 33]. When embedded in β-cyclodextrin triclosan forms a complex and can exhibit antimicrobial action with minimum quantities [34]. Some researchers claim that triclosan inhibits a specific function i.e., lipids synthesis in a bacteria [35]. Others claim that lower levels of triclosan resistance by strains of bacteria shows that triclosan inhibits bacterial cell function in multiple ways. A decrease in the antimicrobial efficiency of triclosan treated material when the material is subjected to repeated home wash cycles has been reported by [36]. One of the greatest concerns regarding triclosan is that when exposed to sunlight, it breaks down into 2, 8-dichlorodibenzo-p-dioxin, a chemical related to other harmful polychlorinated dioxins. Therefore, it has raised a lot of concern in European governments, and its application in consumer products is banned in some countries [37, 38].

2.2 Bound or non-leaching type antimicrobials

The antimicrobial agents that belong to this category are chemically bound to the textile substrate. Hence, the antimicrobial can act only on the microbe that are in contact with the treated textile's surface. By virtue of its binding nature, these antimicrobials are not depleted and therefore potentially may have higher durability than [39]. However, compounds on a treated fabric might get abraded or deactivated with long-term usage and lose their durability [40]. The antimicrobial agents listed under this category are Quaternary Ammonium Compounds (QACs), Polyhexamethylene Biguanide (PHMB), chitosan and N-halamines.

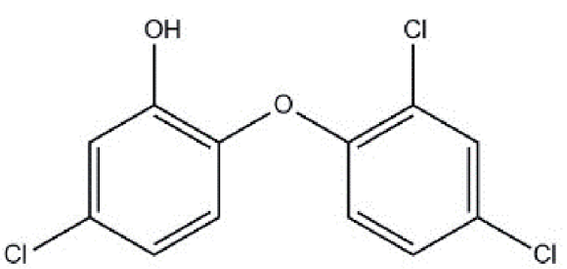


Figure 1.
Molecular structure of Triclosan.

2.2.1 Quaterny ammonium (QAC)

Surface-active agents (surfactants) contain two distinct regions in their molecules: a long chain hydrophobic hydrocarbon tail and a hydrophilic head. Based on the charge of the hydrophilic group, they are classified into cationic, anionic, nonionic, and amphoteric compounds. Among the wide range of these surfactants, the cationic agents (Quaternary Ammonium Compounds or QACs) are known to be the most effective (**Figure 2**). QACs have significant antimicrobial properties and are excellent for deodorization and hard surface cleaning. They are used as biocides in a variety of consumer products, including toothpaste, mouthwash, shampoo, soap, deodorant, etc. The application of QACs as disinfectants goes back to 1936, where Dunn investigated the antibacterial properties of alkyldimethyl-benzylammonium chloride and found the phenol-coefficients against *S. aureus* and S. Typhosa (**Table 1**). The most widely used QACs are monoquaternary ammonium such as alkyltrimethylammonium bromide, and diquaternary ammonium salts such as alkanediyl-α,ω-bis (dimethylalkylammonium bromide). Murugan et al. studied the antibacterial behavior of five novel insoluble bead-shaped, polymer-supported multi-quaternary ammonium salts containing two to six quaternary ammonium groups [41]. The QACs showed excellent antibacterial activity against S.aureus, Klebsiella pneumonia and *Pseudomonas aeruginosa* (P.aeruginosa). Murugan et al. also found that the antibacterial activity increased as the number of QACs in the structure increased [41]. However, there are also reports of bacterial resistance to QACs [42, 43]. Following alkyldimethyl-benzylammonium chloride, other QACs such as Cetyldimethyl-benzylammonium halide and N-(acylcolaminoformyl methyl)pyridinium chloride were studied and were found to have high phenol-coefficients. These solutions were known to be both bacteriostatic and bactericidal, according to the period of exposure and concentration [44].

The attachment of QACs to a textile material is known to be predominantly by ionic interactions between the anionic fiber and the cationic QAC. Therefore, in the case of fabrics that contain sulfonate or carboxylic groups, QACs can be attached to fibers by using an exhaustion dyeing process [45–47]. In the case of synthetic fibers, which contain fewer reactive sites and are quite resistant to antibacterial finishing modifications (such as Nylon 66); dye molecules can act as bridges to bind the functional molecules to fibers [48]. For example, acid dyes can be used to dye the fabric and then QACs can be applied under alkaline conditions. This ionic bonding between the QAC and the dye is relatively strong and provides a semi-durable antibacterial finishing [47, 48]. Hence a dyed fabric can achieve higher add on levels of QACs and antimicrobial efficacy as compared with undyed fabrics [48]. One commercial QAC- based antibacterial textile is Bioguard®. The active antimicrobial agent is 3-trimethoxysilylpropyldimethyloctadecyl ammonium chloride, also known as AEM 5700 or Dow Corning 5700 Antimicrobial agent, which has an MIC = 10–100 mg/L against Gram-negative and Gram-positive bacteria. This

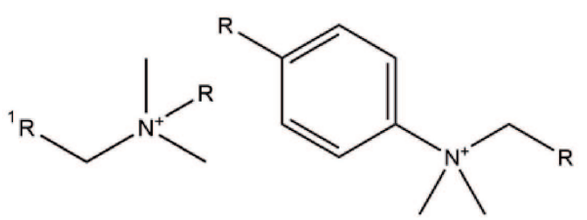


Figure 2.
General molecular structure of Quaterny ammonium (QAC).

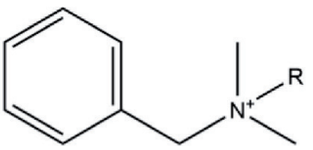
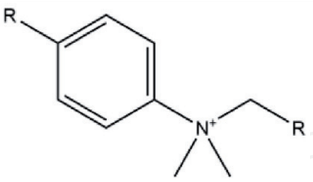
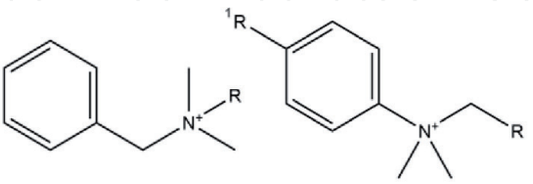
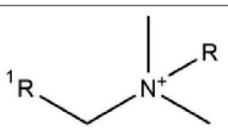
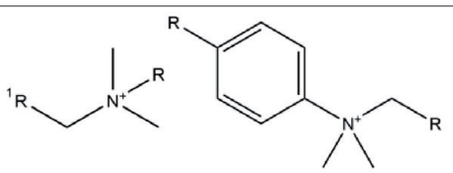
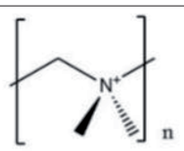
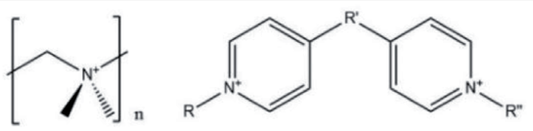
Generation	Compound example	Description
1st		Benzalkonium, alkyl chains, C12 to C18
2nd		Aromatic rings with hydrogen and chlorine, methyl and ethyl groups
3rd		Dual QACs; mixture of alkyl dimethyl benzyl ammonium chloride (lower toxicity)
4th		Dialkylmethyl aminos with twin chains
5th		Synergistic combinations of dual QACs
6th		Polymeric QACs
7th		Bis-QACs with polymeric QACs

Table 1.
Molecular structures and description of the different generations of quaternary ammonium (QAC).

compound is made into aqueous solution and applied by spraying, padding, and foam finishing. During drying, silane forms covalent bonds with the textile, resulting in excellent durability. This compound has been commercially used on nylon, cotton, and polyester. Recently, novel quaternary ammonium functional dyes have been applied on textiles in order to combine antimicrobial finishing and dyeing of textiles in a single step [49–51].

2.2.1.1 Mechanisms of Quaterny ammonium (QAC) antimicrobial action

QACs are active against a broad range of microorganisms such as fungi, Gram-positive and Gram-negative bacteria, and some viruses. QACs have a positive charge on the N atom and inflict a variety of effects on microorganisms, including the disruption of cell membrane, denaturation of proteins, and damage to cell structures. It has been proposed that during the inactivation of bacteria, the quaternary ammonium group remains intact and can retain its antibacterial ability as long as

the QAC is attached to the fibers [48]. When a microbe approaches a QAC treated fabric, the free end of the agent's molecule reacts with the cell wall and causes a leakage of the negatively charged species in the microbe cell. It eventually causes the cell's death [39, 52]. The cationic ammonium group and the negatively charged bacteria membrane are attracted to each other. Consequently, the interactions result in the formation of a surfactant-microbe complex that interrupts all the normal functions of the membrane [53]. QACs affect bacterial DNA, causing a loss of multiplication ability [5]. If the long hydrocarbon chain is bonded to the cationic ammonium in the structure of the QAC, two types of interactions between the agent and the microorganism can occur: a polar interaction with the cationic nitrogen of the ammonium group and a non-polar interaction with the hydrophobic chain. Penetration of the hydrophobic group into the microorganism consequently occurs, enabling the alkylammonium group to physically interrupt all key cell functions [5]. The efficiency of the quaternary ammonium depends on the generation and chain substitution. It is known that the germicidal power increases with an increase in carbon chain length, while the surface activity also increases in the same way [44]. The QACs with 12–18 carbons have been used extensively as disinfectants. The typical dosage is under 1%, and even under 0,1% for some application.

To resume, the quaternary ammonium compounds are membrane active agents, their target site is at the inner (cytoplasmic) membrane in the bacteria (or plasma membrane in yeasts) [8, 44]. One of the mechanisms proposed for the antimicrobial action of QACs is in this sequence:

- Adsorption and penetration of QAC in the microorganism's cell wall
- Reaction with the lipid or protein cytoplasmic membrane, which will disorganize the membrane
- The leakage of low molecular weight intracellular material
- Degradation of nucleic acids and proteins
- Wall lysis which is caused by the autolytic enzymes.

Without detailing the studies carried out on the toxicity of quaternary ammoniums, different experiences were carried out on their ocular toxicity [54, 55], contact dermatitis [56], their skin sensitizer (human contact allergen) and asthma [57, 58]. Quaternary ammonium compounds are known to cause occupational asthma. It was found that nurses exposed to a class of QAC and all exhibited early or delayed asthma symptoms when handling disinfectant solutions containing QAC. The same study was done with products lacking in QAC and the results were negative [59]. These results have been confirmed by a multitude of studies [57–59]. In parallel, it has been reported that repeated occupational exposure after handling QACs as powders or solutions could cause sensitization [60]. In conclusion, the studies above all confirm the link between prolonged exposure to quaternary ammonium compounds and asthma. However, regarding ocular and dermal irritation, it seems that the quaternary ammonium compounds allergenicity is likely to be related to the compound's solubility. Apparently, no quaternary ammonium compounds can be regarded as allergens. In most of the studies that classify these compounds as irritants/allergens, the lipid or water-soluble compounds have been studied, while the non-soluble QACs certainly do not have the same properties.

2.2.2 Polyhexamethylene biguanide (PHMB)

PHMB is a hetero disperse mixture of polyhexamethylene biguanide (**Figure 3**). Polyhexamethylene biguanide (PHMB, commercially known as Vantocil) is a broad-spectrum antibacterial agent with low toxicity, having an MIC = 0.5–10 ppm. It has been previously used as a disinfectant in pool sanitizers, mouthwashes, wound dressings, and in the food industry. PHMB can disrupt the integrity of cell membranes [61].

The halide form of PHMB i.e., polyhexamethylene biguanide hydrochloride is applied on cellulosic materials [62]. PHMB is found to form hydrogen bonds with cellulosic fibers. With the increase in the concentration of PHMB there is a dominant increase in hydrogen bond formation between PHMB and fibers [63]. When the fabric treated with PHMB is exposed to a bacterium, the biocide interacts with the surface of the bacteria and is transferred to the cytoplasm and cytoplasmic phospholipids in the bacterial membrane. This biocide is positively charged, and therefore it mainly reacts with negatively charged species and includes aggregation, leading to increased fluidity and permeability. This results in the leakage of inner material from the outer membrane and eventually causes death of an organism [52].

2.2.3 Regenerable N-halamines and Peroxyacids

N-halamines are heterocyclic compounds containing one or two covalent bonds formed between nitrogen and halogen [64]. N-halamines contain one or more nitrogen-halogen covalent bonds formed by the chlorination or bromation of imide, amide or amine groups. The halogen, which is usually chloride, is replaced with hydrogen in presence of water or chloroform and acts as biocide (**Figure 4**) [65]. By using chlorine-containing N-halamine compounds, durable antibacterial finishing can be achieved on textiles. N-halamines are broad-spectrum disinfectants, which have been used previously for water treatment. Their antibacterial activity is known to be due to the oxidative properties of halamine bond (N-Cl). In order to kill the bacteria, N-Cl will be transformed to N-H, which can be recharged with chlorine (during laundering, by using bleach). The product of the reaction is reversible, meaning the N-halamide can be regenerated with the presence of chlorine compound. This function is found in hypochlorite, commonly found in bleach solution. The regeneration with bleach can be done during the washing process. This novel regenerable method was first proposed by Sun and Xu for the treatment of cotton fabric [66]. Since then, many different heterocyclic N-halamines have been applied on polyester, nylon, keratinous fibers, and cotton through covalent bonding. In all these studies, it was demonstrated that regenerable and durable antibacterial activity can be achieved by recharging the fabric in aqueous chlorine solutions.

N-halamine compounds, of which N-chloramine is one form, can provide instant and complete kill of a broad-spectrum of microorganisms. The antibacterial property is based on active chlorine, Cl^+. Two mechanisms can be used to explain

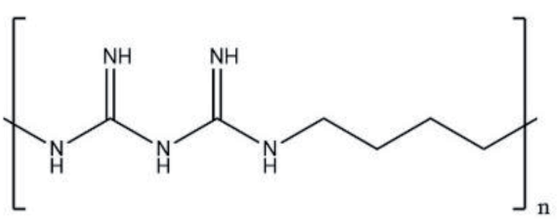


Figure 3.
Molecular structure of Polyhexamethylene biguanide (PHMB).

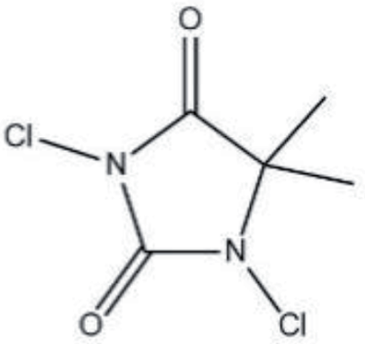


Figure 4.
Molecular structure of N-halamines.

the antibacterial activity of N-chloramine. One mechanism is that free chlorine is released into water and then forms HClO or ClO^-. The other is that chlorine binds directly to acceptor regions in bacteria and greatly influences their enzymatic and metabolic processes [64]. It was found that the antibacterial activity mainly attributed to the second mechanism because the dissociated chlorine is limited [67]. N-halamines possess stability that is suitable for long-term use, storage and regeneration. N-chloramine can be achieved by the reaction between sodium hypochlorite solution and imide, amide or amine groups. N-halamines have been used in water treatment and incorporated into cellulose-containing fabrics, polyester fibers and polyamide [68–70]. Although no research has directly addressed N-halamine in wound dressing, it has been grafted onto fibers or fabrics so it may be used in wound dressing [69, 70].

Halamine can be applied on different textile including cellulose, polyamide and polyester fibers [71–74]. It has also been found to have extraordinarily durable biocidal functions in a series of laundering tests [75]. However, N-halamine materials are found to be decomposed upon exposure to ultraviolet irradiation as in direct sunlight [76]. The main problem with N-halamines was that they result in a significant amount of absorbed chlorine (or maybe other halogens), which can remain on the fabric surface, resulting in unpleasant odor and fabric discoloration. The use of bleach and the presence of strong oxidizing degrade the dye on the textile, which leads to discoloration of the textile. This antimicrobial technology is best used on bleach resistant textile. One method known to resolve this problem is using a reduction step to remove the residual unbounded halogen from the surface of fabric [75–79]. An alternative antibacterial finishing agent is known to be peroxyacids (such as peroxy acetic acid, which is extensively used in hospitals.) Peroxyacids should convert to carboxylic acid in order to deactivate bacteria, but can be regenerated by reacting with an oxidant (such as hydrogen peroxide). Despite the stability of the peroxyacids on the fabric during prolonged periods, the antibacterial activity reduces largely after a number of washing and recharging cycles [73, 74].

2.2.4 Chitosan

Chitosan is derivatized by the deacetylation of Chitin, the main component of shrimp, crab, and lobster shells. Chitin, a poly (β-(1–4)-N-acetyl-D-glucosamine) is a natural polysaccharide. Chitin is synthesized by many living organisms. Chitin is the second most abundant polysaccharide in nature after cellulose [80]. When chitin is acetylated to at least about 50%, then it is called chitosan [28]. Chitosan (**Figure 5**) contains three reactive sites including a primary amine and two primary or secondary hydroxyl groups per glucosamine unit. As a result, it is readily

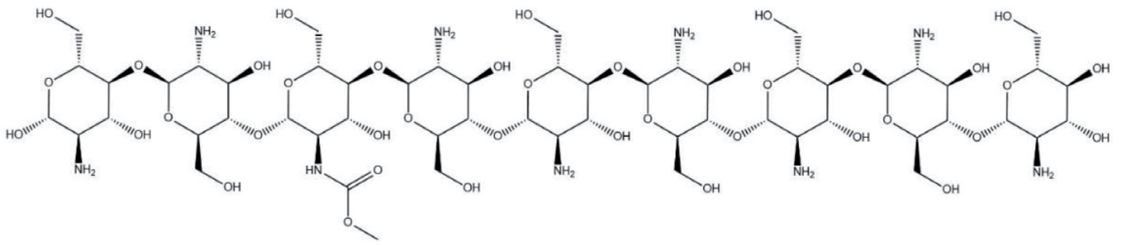


Figure 5.
Molecular structure of chitosan.

subject to chemical modification. The structural characteristics of chitosan mimic glycosaminoglycan components of the extracellular matrix, so the biocompatibility, biodegradability, antibacterial, hemostatic and antioxidant activities and muco-adhesive properties impart versatility [81]. Chitosan's good antibacterial activity along with its biodegradability, biocompatibility, and most importantly nontoxicity makes it an ideal biocidal agent in food science, pharmaceuticals, medicine, and textile applications. Despite all these advantages, chitosan lacks good solubility above pH 6.5. Its applications in a commercial context are not as wide as might be expected [82]. One of the potential problems with an effective chitosan based anti-microbial agent is that chitosan is insoluble in water and possesses high molecular weight. The high molecular weight increases the viscosity of the medium and causes detrimental effect on the hand and feel of the fabric [83]. Chitosan can be used to spin antimicrobial fibers or as a finishing agent for surface modification. Therefore, researchers are exploring chitosan derivatives that are soluble in water over a wide pH range for expanding the chitosan applications.

2.2.4.1 Chitosan derivatives

Given that chitosan does not dissolve in aqueous media at neutral and alkaline pH's and its antimicrobial activity likewise is not particularly good in neutral or alkaline solutions, so there is many causes to chemically modify chitosan. These modifications were made with the aim of proposing more soluble chitosan derivatives better suited for textiles. Recent researchers reported that chitosan derivatives have better water solubility, antibacterial and antioxidant properties [84]. Chitosan can be modified to include quaternary ammonium groups, alkyl and aromatic groups, substituents having free amino or hydroxyl groups, carboxyalkyl groups and amino acids and peptides [85]. And different applications have been found for these chitosan derivatives. Among the derivatives of chitosan we cite: Carboxymethyl Chitosan, N,N,N,-trimethyl chitosan (TMC) and Chitosan nanoparticles (CSNP).

The modification of the structure of chitosan by the addition of carboxymethyl in the structure of chitosan allows the manufacture of carboxymethyl chitosan (CMC). Compared to chitosan, CMC is characterized by high solubility at neutral and alkaline pHs. This modification does not affect its characteristic properties [86]. In addition, CMC has superior antimicrobial activity, biocompatibility and safety for humans. Usually, there are O-CMC, N-CMC, N, singlet O and N, N-dicarboxymethyl chitosan that have different chemical structures (**Figure 6**). For antimicrobial properties, the antimicrobial activity of different types of CMCs against *E. coli* has been shown to increase by converting NO-CMC < Chitosan <O-CMC due to the reduced number of protonated amino groups in NO-CMC [86]. And against *S. aureus*, O-CMC and N-CMC also have improved antimicrobial properties [86–88].

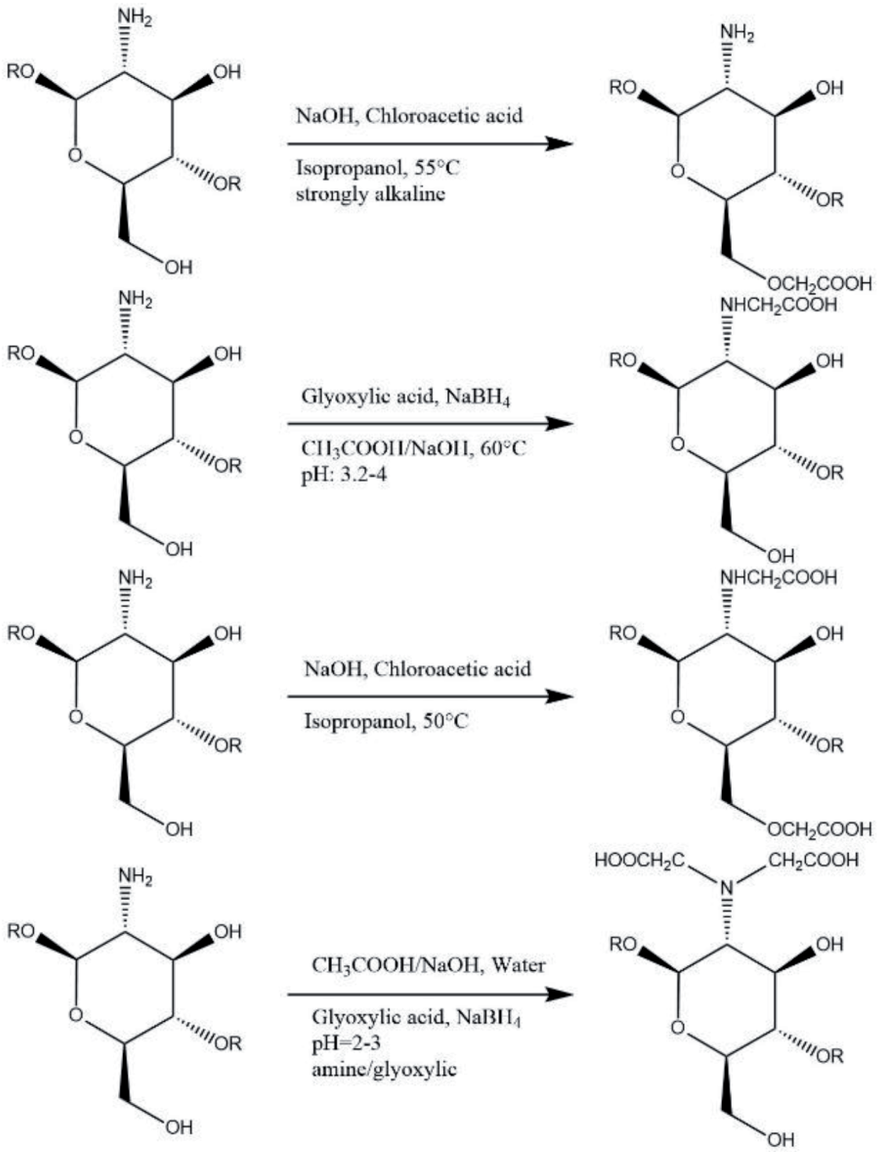


Figure 6.
As example, some carboxymethyl derivatives of chitosan [86].

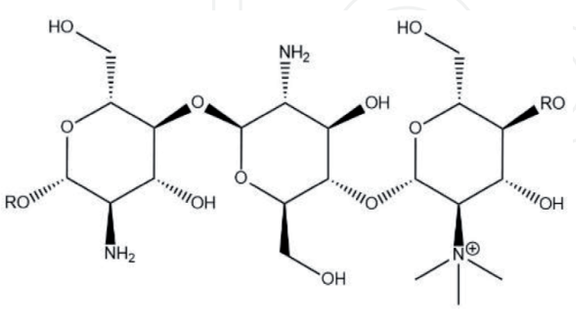


Figure 7.
Molecular structure of N, N, N-trimethyl chitosan [89].

The second chitosan derivative fairly presented in the literature is TMC (**Figure 7**). It is a partially quaternized derivative [89]. It is obtained by nucleophilic substitution of the primary amino group in position C-2 by a quaternary amino group [89]. This modification facilitates the aqueous solubility of TMS at neutral and basic pH due to the presence of a permanent positive charge independent of pH (the quaternary amino amino groups) [90, 91]. This high positive

charge density is responsible for the high antibacterial performance reported for TMC compared to Chitosan [92–94]. It is essential to point out that this modification takes place under alkaline conditions using sodium iodide as catalyst and N-methyl-2-pyrrolidone as solvent [95]. In addition, it can take place by reaction with dimethyl sulfate [96, 97] or dimethylformamide [98].

Among the derivatives of Chitosan, which have a higher antimicrobial activity than the chitosan solution, there are the chitosan nanoparticles (CSNP). For the moment, there is no clear explanation for this high efficiency, but one of the hypotheses given is based on the high specific surface area of nanoparticles as well as their better affinity for the microbial cell wall [99]. Unlike TMC, CNSPs are prepared with simple methods, without organic solvent or high shear force. Among these methods there are: emulsion crosslinking, coacervation (precipitation), ionic gelation, spray drying, microemulsion, diffusion of emulsifying solvent and polyelectrolyte complex [100]. The degree of deacetylation of chitosan and its molecular weight are factors that affect the formation and size of CNSPs [101].

2.2.4.2 Mechanisms of chitosan's antimicrobial action

Chitin is a film-forming polymer with antibacterial and fungi-static property. It triggers the defensive mechanism in host inducing certain enzymes like phytoalexins, chitinases, pectinases, glucanases, and lignin in plants [28]. Chitosan and its derivatives have been studied as antimicrobial agents against bacteria, fungi and viruses, in experiments involving in vivo and in vitro interactions with chitosan in different forms (solutions, nanoparticles, films, fibers and composites). Chitosan can react in two different mechanisms, killing or inhibiting the growth of microorganisms (biocide or Biostatic). However, its action often takes place without distinction between activities [102]. The antimicrobial performance of Chitosan or one of its derivatives depends largely on its molecular structure and its properties such as molecular weight [103], the degree of deacetylation and its water solubility [104–108]. In addition, its pH and its concentration in solution affect its effectiveness against microorganisms [109]. Chitosan has MIC of 0.05–0.1% (w/v) against most common kinds of bacteria. The mode of its antibacterial action is not yet fully understood [110–112], but it is possible that the amine groups provide positive charges which can react with the negatively charged surface of microbes; therefore, they can change the cell permeability, which finally leads to intracellular substance leakage [113, 114]. Chitosan acts primarily as a disruptor of the outer membrane and not as a penetrating agent. Using transmission electron microscopy, impaired membrane function was demonstrated by shrinkage, implying that water and ion leakage had occurred. However, other studies have proposed a mechanism by encapsulation where chitosan forms a polymeric substance around the bacterial cell preventing nutrients from entering the cell and its subsequent death [115]. In addition, a third mechanism based on metal chelation, removal of spore elements and binding to nutrients essential for microbial growth is proposed. This mechanism is based on the strong binding capacities known for chitosan to metals. This absorption of cations occurs by metal chelation favored by the amino groups present in the chitosan molecules [115]. The efficiency of this mechanism depends largely on the pH of the medium. A high pH favors this absorption mechanism by the fact that the amino groups of the chitosan will not be protonated under these conditions. This will allow the pair of electrons on the nitrogen atom to be available for donation to metal ions. Some studies have assumed that the metal can behave as an electron acceptor which connects via $-NH_2$ functions to one or more chitosan chains, forming bridges with hydroxyl groups [116].

2.2.4.3 Chitosan and chitosan nanoparticles (CSNP) on textiles applications

Even though, chitosan has already been utilized for the treatment of fibrous materials, a comprehensive research on their use for antimicrobial functionalization of viscose fibers for development of modern medical textiles for applications in medical devices is still missing. There is a lack of information regarding the behavior of different chitosans in contact with the cellulose materials. In addition, in-depth knowledge of their physical, chemical, and biological properties is missing [117, 118].

For textiles, chitosan and its derivatives could constitute one of the products that can be used for the finishing processes [116]. In addition, they could be used for the production of raw materials such as chitin and chitosan fibers [116]. The latter are widely used, alone or in admixture with other products such as viscose, in the medical field for the manufacture of non-woven, which can be used for dressings, or as a carrier for medicaments in the form of hollow fibers [119, 120]. Microbiological tests showed antimicrobial activity, no cytotoxicity was detected for a chitosan-polypropylene nonwoven [119]. Viscose tampons treated with chitosan were utilized for maintaining the physiological pH of vagina and acting as moisturizing agent, while simultaneously providing antimycotic and antibacterial activity [121]. Textiles treated in such a way were effective against gram-negative and gram-positive bacteria [122]. Some studies have shown that chitosan-based products provide rapid healing and less dense skin lesions compared to standard products [122–124]. By way of example, it has been found that the treatment of cotton with a carboxymethyl chitosan derivative at a concentration of 0.1% provides antibacterial protection against *E. coli* and *S. aureus*, as well as an improvement in the wrinkle recovery. Alternatively, core-shell particles based on chitosan (shell) and poly (n-butyl acrylate) (PBA) (core) have been designed as a novel antibacterial coating for textiles [125]. These particles applied to a cotton fabric showed an antibacterial performance of over 90% against *S. aureus*. The application of chitosan on cotton takes place without the need for a crosslinking agent. Studies have shown that an intermolecular hydrogen bond between the hydroxyl groups of cotton and the amino groups of chitosan [125]. Antibacterial efficacy against *S. aureus* was examined for chitosan nanoparticles loaded with silver (CSNP) applied to polyester. An efficiency of 100% was obtained. This efficacy is attributed to the synergistic effect of silver and CSNP [126].

2.2.5 Natural antibacterial

In addition to chitin and chitosan, antibacterial performance has been identified for other naturally occurring products such as honey. The latter and because of its antibacterial activity allows the treatment of certain burns [127–129]. This antibacterial performance, against certain large bacteria-infecting wounds such as *S. aureus* known for its resistance to methicillin, is attributed to the presence of certain molecules such as hydrogen peroxide and bioflavonoids. The latter have the capacity to inhibit the synthesis of nucleic acids [130].

At the same time, we must not forget the products of plant origin (*Aloe Vera*, tea tree oil and eucalyptus, extracts of neem, grapefruit seeds and tulsi leaves, etc.) which have a high antibacterial performance. This efficiency is attributed to their content in certain phenol type products (simple phenols, phenolic acids, quinines, flavonoids, flavones, tannins and cumarins), terpenoids, essential oils, alkaloids, lectins, polypeptides and polyacetylenes [131]. In addition to their antibacterial performance, these components are known for their antioxidant properties. The combination of these two functionalities constitutes an important advantage

for biomaterials that can be used for medical applications such as dressings. It ensures a reduction in the formation of reactive oxygen species, which are strongly involved in the pathogenesis of injury. Usually these species cause the formation of biomolecules (lipids, proteins and nucleic acids) at the level of injury as well as the depletion of mitochondrial DNA in human skin [132]. Among the powerful antioxidants are the flavonoids, which are used as anti-inflammatory, antimicrobial and anticancer agents [132]. But, durability and resistance to washing remains the weak point for the antimicrobial finish based on natural agents. This weakness results from their difficulty in forming bonds with textile materials [133]. Certain methods have been developed to increase this durability. Among these methods, mention may be made of microencapsulation [133–135], the use of a crosslinking agent [131] and the immobilization of bioactive liquids in sol–gel matrices are also described in the literature [135]. For example, antimicrobial activity against *S. aureus* has been recorded for cotton fabrics treated with *Aloe Vera* extract by a dry drying process. This effectiveness was sustainable even after 50 wash cycles [136].

3. Standard tests for antimicrobial activity

The antimicrobial efficacy of textiles could be characterized by different methods of analysis. These methods are standardized and divided into two categories: 1) qualitative, such as AATCC TM147, AATCC TM30 (American Association of Textile Chemists and Colorists Test Method), ISO 20645, ISO 11721 (International Organization for Standardization) and SN 195920, SN 195921 (Swiss standard) and 2) quantitative, such as AATCC TM100, ISO 20743, SN 195924, JIS L 1902 (Japanese industry standards) and ASTM E 2149 (or its modification).

Qualitative methods are characterized by their speed and simplicity. They are mainly based on the agar diffusion test. As diffusion through agar occurs at different rates depending on the textiles and the nature of the antimicrobial agents used, this category of methods is not suitable for all types of textiles. Some differences could be identified between the different qualitative methods. As an indication, we can mention that the textile is laid on an inoculated agar plate for AATCC TM147. While it is place between two agars plates, with one side inoculated for ISO 20645. Usually the qualitative method has an incubation period. After this period (24–48 h) depending on the type of microorganisms tested, the plates are examined for bacterial growth directly underneath the fabric and around its edges (zone of inhibition). The appearance of the zone of inhibition depends on the ability of the antimicrobial agent to diffuse into the agar and it is binding to the textiles. The appearance of a zone of inhibition and its size are indicative of the rate of release of the active agent and its antimicrobial efficacy. It is important to specify that zone of inhibition does not necessarily imply that microorganisms have been killed; they might have only been prevented from growing. By qualitative methods, the efficacy of different agents cannot be compared [137].

On the other hand, quantitative methods can be used for the majority of antimicrobial agents and textile supports. However, they require a longer time compared to qualitative methods. In addition, they are more expensive because they involve a real count of the microbes to measure the antimicrobial effectiveness. This method of measurement makes it easier to compare the effectiveness of different antimicrobial agents on the same textile support, for example [138–141]. Quantitative methods are much more specific depending on the mechanism of action of the antibacterial agent. For example, ISO 20645 can be tested with only leaching types because the configuration does not allow observation under the textile [141]. AATCC TM100 and JIS L 1902 can be tested with leaching and non-leaching type's antimicrobial. These

methods (AATCC TM100, ISO20743 and JIS L 1902) are based on similar principle: specified amount (weight, size, and surface area) of sample swatches or substrate are inoculated with a specified number of microorganisms [142]. The inoculum is put in contact with the treated surface via three different methods: absorption, transfer and printing (ISO 20743). The absorption method uses an inoculated broth with a standardized species and concentration. The broth is absorbed by the textile sample. The sample is incubated in different condition depending on the method, to promote the bacterial growth. This method allows testing a leaching, non-leaching or a combination of antibacterial textile as well as bacteriostatic and bactericidal. However, this method is not recommended for textile with hydrophobic treatment or low absorption capacities [143]. While, the transfer method uses an agar plate who is inoculated with the tested bacteria. The contaminated plate is put in contact with the textile for 60 seconds, and after the sample is incubated. This method is used to replicate the contact of the antimicrobial textile with a contaminated surface. Whereas, the printing method apply the bacteria via a printer. This method allows faster incubation time (1 to 4 h, ISO 20743) and faster sample preparation with the automated printer (ISO20743). Finally, the dynamic shake flask method (ASTM E 2149 (or its modification) is particularly appropriate for non-leaching antimicrobials whilst the dynamic contact conditions are applied to the samples [140]. It can be used to assess the activity of the antimicrobial textile as a qualitative test. This method has been used for testing antimicrobial activity of cotton fabrics (or cellulose fibers) treated with the nanoparticles [144, 145], as well as functionalized wool [146], cotton and viscose fibers coated with chitosan [147, 148], and some other fabrics.

Once the microorganism and incubation application protocol are applied according to the desired method, the microorganism count will take place via two different techniques: the plate count method and the luminescence method. The count plate method consists of recovering the microorganism from the broth by re-plating and the number of surviving organisms. The number of colonies forming unit (CFU) is counted and the bacteria concentration is obtained by multiplying the dilution rate. The ATP concentration is quantified via a spectrophotometer according to the luminescence method. This measurement will be compared with a calibration curve prepared according to the ATP standard. The quantification of the ATP of the inoculum is carried out before and after exposure to the antimicrobial treatment. The number of surviving organisms is counted as CFU and results are usually presented as percentages or log10 reduction in contamination relative to the initial inoculum of microorganisms or the untreated control.

It should be noted that antimicrobial analysis methods are quite sensitive to contaminate. For this reason, tests are usually done under tightly controlled conditions to ensure reproducibility of results. However, carrying out tests in such a standardized environment does not reflect the reality of using textiles treated with antimicrobial agents [137]. Another factor that affects these tests is the efficiency of microbial extractions from the sample tissues. In addition, the absence of an absolute standard of effectiveness facilitates changes in the protocols applied creating inconsistencies between laboratories at national and international levels. Taking into account all the factors affecting the effectiveness of antimicrobial tests, certain additional methods are applied in complementarity. These methods include colorimetric analyzes [149], viability test [150], viability staining and microscopy [151], and fluorescent staining coupled with flow cytometry [152]. Despite the advancements made to date, the poor reproducibility of test results is the Achilles point of these tests. Over time, some attempts to establish a correlation between the different analytical techniques have taken place. For example, AATCC TM147 and JIS L 1902 were found to give the same result for a textile sample with a non-leaching antimicrobial [137]. Nevertheless, the strong differences are always an obstacle.

4. Conclusion

In conclusion, the microorganism presence on textile can be eliminated or the growth can be slowed by treating with a variety of antimicrobial agent. Multiple antimicrobial families were presented in this chapter, including synthetic and natural chemicals (**Table 2**). Textiles are susceptible to microorganism growth because of the structure and the ability to retain moisture of the textile. Therefore, the microorganism growth can generate multiple undesired consequence, such as hosting and transmitting harmful microbe, creating odor, mold, degradation, discoloration and biofouling for example. Textile can be treated with antimicrobial agent to reduce, slow or eliminate the microbial growth and spread. The antimicrobial was categorized in two types in this chapter, leaching and non-leaching. The non-leaching types are bound to the textile and react with the microbes upon direct contact. On the other side, the leaching types release antimicrobial in the environment at a controlled rate to disrupt the microorganism at proximity of the textile. A summary of the common reagents discussed in this chapter is gathered in the **Table 3**.

The antimicrobial efficiency, the durability and skin compatibility of the treated textile must be assessing during the development of an antimicrobial treatment to minimize the risk. The antimicrobial activity testing can be categorized by quantitative and qualitative method. The qualitative method is useful for routine quality control and for the screening of multiples iterations during the development of a product, such as determining the wash durability. However, this method can lead to subjective determination. Instead, the quantitative method eliminates the possible subjectivity with plate count and luminescence technique. In addition, the skin should not be harmed be the treated textile. The safety for the skin can be evaluated with cytotoxicity test to human cell and irritation test in-vitro and in-vivo. The properties of the antimicrobial should be assessed before commercialization of an antimicrobial treated product.

Mechanism	Reagent	Fiber	Remarks
Leaching	**Metals (silver)**	Nylon, Wool, Polyester and Regenerated- cellulose	Slow release, durable, depletion of Ag might occur
	Triclosan	Polyester, Nylon, Acrylic, Polypropylene and Cellulose- acetate	Breaks down into toxic dioxine, large amount needed, bacterial resistance, banned in some European countries
Non-Leaching	**QACs**	Cotton, wool, Nylon, Polyester and Acrylic	Very durable, covalent bonding, possible bacterial resistance
	PHMB	Nylon, Polyester and Cotton	Large amount needed, bacterial resistance
	N-halamine	Wool, Nylon, Cotton and Polyester	Requires regeneration, unpleasant odor from residual Cl
	Peroxyacids	Cotton and Polyester	Poor durability, requires regeneration
	Chitosan	Wool, Polyester and Cotton	Low durability, adverse effect on fabric handle

Table 2.
Conventional reagents used in the antimicrobial finishing of textiles.

Test	Method	Title	Principle	Antimicrobial type	Uses
ASTM E2149	20	Standard Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions	Dynamic shake flask test	Non-leaching type	Qualitative: Screening, routine quality-control
AATCC 147	Parallel Streak	Antibacterial Activity of Textile Materials: Parallel Streak	Zone diffusion assay: Agar	Leaching-types	Qualitative: Screening, routine quality-control
AATCC 100	—	Test Method for Antibacterial Finishes on Textile Materials: Assessment of	Cell suspension intimate contact test	Leaching and non-leaching	Quantitative
XP G 39–010	—	Propriétés des étoffes - Étoffes et surfaces polymériques à propriétés antibactériennes - Caractérisation et mesure de l'activité antibactérienne	Cell suspension intimate contact test	Leaching and non-leaching	Quantitative
JIS L 1902	Absorption method	Testing Method for Antibacterial Activity of Textiles Quantitative Test	Cell suspension intimate contact test	Leaching and non-leaching	Quantitative
JIS L 1902	Transfer method	Testing Method for Antibacterial Activity of Textiles Quantitative Test	Transferred Agar plate contact test	Leaching-types	Quantitative
JIS L 1902	Printing method	Testing Method for Antibacterial Activity of Textiles Qualitative Test	'Dry' inoculum intimate contact test	Non-leaching	Quantitative
JIS L 1902	Halo Method	Testing Method for Antibacterial Activity of Textiles Qualitative Test	Zone diffusion assay: Agar	Leaching-types	Qualitative: Screening, routine quality-control
ISO 20645	Agar diffusion plate test	Textile fabrics — Determination of antibacterial activity — Agar diffusion plate test	Zone diffusion assay: Agar	Leaching-types only	Qualitative: Screening, routine quality-control
ISO 20743	Absorption Method	Textiles - Determination of antibacterial activity of antibacterial finished products: Absorption Method	Cell suspension intimate contact test	Leaching and non-leaching	Quantitative

Test	Method	Title	Principle	Antimicrobial type	Uses
ISO 20743	Transfer Method	Textiles - Determination of antibacterial activity of antibacterial finished products: Transfer Method	Cell suspension intimate contact test	Leaching-types	Quantitative
ISO 20743	Printing Method	Textiles - Determination of antibacterial activity of antibacterial finished products: Printing method	'Dry' inoculum intimate contact test	Non-leaching	Quantitative

Table 3.
Comparison of antimicrobial test method for textile.

Antimicrobial resistance should also be a concern when developing an antimicrobial treatment for a textile because of the large quantity of antimicrobial agent required achieving the antimicrobial activity and durability. The risk/reward should always be considered before applying antimicrobial product to a textile. The risk of antimicrobial resistance can be minimized. First off, the antimicrobial should not come close to the minimal inhibitory concentration (MIC) of the treatment during the useful life of the product to guarantee an effective product. The MIC of the antimicrobial product can be reached because of poor wash durability of the antimicrobial product. Second of, the synergy, mechanism of different antimicrobial product can be combined to reduce the resistance of a gene to a pathway. A complex antimicrobial mechanism is believed to be more efficient and more complex for the microbe to develop a set of successful mutated gene against the antimicrobials [153].

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) (COVPJ 553781 - 20), and the MEI-Québec (Ministère de l'Économie et de l'Innovation).

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