Review

Antimicrobial Agents in Healthcare

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Abstract:

The increasing resistance of microorganisms highlights the need for new antimicrobial agents in healthcare, particularly for preventing infections on medical surfaces and implants. Promising approaches include quaternary ammonium compounds, chitosan, and graphene. Quaternary ammonium compounds are effective against a wide range of bacteria and viruses; however, their synthetic nature and environmental impact pose limitations, as well as the risk of developing resistance. Chitosan is a natural biopolymer with biocompatibility, antiviral, and antifungal properties; however, its poor solubility limits its use, necessitating chemical modifications. Graphene and its derivatives exhibit exceptional mechanical strength, large surface area, and antimicrobial activity, enabling effective prevention of biofilm formation, although their efficacy depends on specific usage conditions. Combining these materials with advanced technologies opens opportunities for developing smart, biocompatible, and environmentally friendly antimicrobial solutions that can complement or replace conventional antibiotics. Further research is essential to enhance efficacy, reduce toxicity, and increase practical applicability in clinical practice.

Keywords: Antimicrobial agents; Quaternary ammonium compounds; Chitosan; Graphene; Medical implants; Biocompatibility.

1. Introduction

The need to design new antimicrobial agents in healthcare is becoming increasingly urgent, both to prevent healthcare-associated infections and to address the growing development of bacterial resistance to existing drugs. The increasing spread of resistance to antibiotics and other antimicrobial substances poses new challenges in the treatment of infections, potentially leading to prolonged hospital stays, higher mortality rates, and increased healthcare costs (Agarwalla et al., 2019). Therefore, the development of new agents is essential for maintaining the effectiveness of therapeutic options and controlling the spread of resistance. Microorganisms that develop antibiotic resistance can spread rapidly, rendering treatments ineffective and posing a serious threat to public health. At the same time, the development of new antibiotics is slow and time-consuming, resulting in a lack of effective solutions when existing drugs fail. Consequently, there is growing interest in natural compounds, which may offer new opportunities to combat resistant bacteria. Natural products have historically played a crucial role in drug development. Over the past 40 years, more than half of newly developed antibacterial drugs have been derived from natural or semi-natural sources, while only about 22% have been purely synthetic (Confederat et al., 2021). Two broad approaches are used to create antimicrobial surfaces: prevention of microbial adhesion and contact-based inactivation. The first approach often involves superhydrophobic surfaces inspired by nature, designed to prevent microbial attachment. However, these strategies are relatively complex, mostly limited to flat surfaces, and their long-term durability remains uncertain. The second approach encompasses a wide range of contact-based inactivation strategies, all of which rely on chemical surface modification to achieve specific bactericidal and virucidal effects (Artusio et al., 2024). Bacterial species of particular interest are those capable of acquiring resistance through various mechanisms of genetic transfer, such as transformation, conjugation, transduction, and mobile genetic elements. Notable examples include *Staphylococcus aureus*, *Enterococcus* spp., *Pseudomonas aeruginosa*, members of the *Enterobacteriaceae* family, and *Acinetobacter* spp. (Confederat et al., 2021).

2. Design of Review

The review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, where applicable. A systematic literature search was carried out using PubMed, Scopus, and Web of Science, covering the period from 2007 to 2025. The search strategy included the following keywords: antimicrobial agents, quaternary ammonium compounds, chitosan, graphene, medical implants, and biocompatibility.

3. Quaternary Ammonium Compounds

Quaternary ammonium salts (QAS) are widely present in nature and play an important role in various biological processes. In addition to their natural occurrence, they are frequently synthesized chemically. Due to their diverse chemical structures and biological properties, QAS are widely used in industrial and medical applications, including disinfectants, pharmaceuticals, DNA carriers, preservatives, biocides, herbicides, and fungicides. An important aspect of their function is their ability to reduce microbial adhesion to both biotic and abiotic surfaces, thereby contributing to the eradication of biofilms formed by pathogenic microorganisms. These biofilms are often resistant to conventional drugs and disinfectants, and the use of QAS can help prevent infections and reduce the risk of disease transmission (Obłąk et al., 2021). Common disinfectants used in routine cleaning are largely based on quaternary ammonium compounds, halogens, and phenols. However, in some cases, their effectiveness against pathogenic bacteria is limited, as it depends on factors such as contact time, pH, temperature, and the type and quantity of microorganisms present (Santoro & Izzo, 2024). The chemical structure of QAS, such as alkyl chain length, the type of counterion, and the linking group, strongly influences their physicochemical properties and biological activity. For example, by integrating into microbial cell membranes, QAS can inhibit key enzymatic activities and disrupt intracellular transport, leading to reduced growth or cell death. However, their widespread use in commercial products raises concerns about the development of microbial resistance not only to QAS but also potentially to antibiotics (Obłąk et al., 2021). Polymeric surfaces containing QAC exhibit low hemolytic activity, making them suitable for safe and effective use in various

applications. These include surgical and medical implants, wound dressings, industrial equipment, water treatment systems, and food packaging, where they help reduce the risk of bacterial contamination (Santoro & Izzo, 2024). Based on structural complexity, QAC can be classified into mono-QAC (one quaternary nitrogen atom), bis-QAC (two quaternary nitrogen atoms), and poly-QAC (multiple quaternary nitrogen atoms) (Mohapatra et al., 2023; -Ortega et al., 2025). The number of quaternary nitrogen centers significantly affects antimicrobial efficacy, along with factors such as the length and nature of alkyl substituents and the presence of functional groups. It is also important to note that QAC has been detected in wastewater and sewage sludge worldwide, often at variable and sometimes high concentrations, particularly in raw sewage and industrial effluents. Although regulatory measures have reduced the levels of certain compounds, a marked increase in QAC concentrations and loads in wastewater was reported during the COVID-19 pandemic, reflecting increased disinfectant use and limited removal efficiency in treatment plants. QAC enters wastewater treatment systems via sewage networks; however, a portion can pass through treatment processes and be released into aquatic environments. It is estimated that approximately 75% of QACs enter treatment plants annually, while around 25% are directly discharged into the environment. Their removal primarily occurs through adsorption onto activated sludge, while biodegradation plays a lesser role, allowing QAC to persist and accumulate in surface waters. Concentrations in effluents and surface water typically range from below 1 to approximately 100 μg/L, while levels in raw wastewater can be significantly higher (Mohapatra et al., 2023). Environmental concerns regarding QAC arise from their potential to promote antibiotic resistance and their role as precursors in the formation of disinfection by-products. Due to slow microbial degradation and limited photolysis, these compounds can accumulate, particularly in sediments. Although detected concentrations are unlikely to pose acute risks to aquatic ecosystems, their cumulative presence may be significant in terms of resistance development and by-product formation (Pati & Arnold, 2020).

3.1. Broad-spectrum antimicrobial activity of QAC

The antibacterial activity of QAC is primarily based on its positive charge, which enables electrostatic interaction with negatively charged phospholipids in bacterial membranes. After initial binding, the hydrophobic alkyl chain inserts into the lipid bilayer, leading to reduced membrane fluidity, disruption of membrane integrity, impaired osmoregulation, and ultimately cell damage and death. As with antibiotics, resistance mechanisms against QAC have also developed over time. Prolonged or repeated exposure can lead to changes in membrane structure, the emergence of mutated enzymes, activation of alternative metabolic pathways, and increased expression of genes associated with biofilm formation and efflux pumps. As a result, microorganisms may develop resistance to QAC, including the ability to metabolize or degrade these disinfectants (Belter et al., 2022). The effectiveness of antibacterial activity is also influenced by material properties, particularly in polymeric surfaces. Key factors include surface charge, molecular size, hydrophilicity, and environmental conditions such as temperature and pH. Polymeric surfaces containing QAC carry a positive charge, enabling strong interactions with negatively charged bacterial cells. This negative charge originates from teichoic acids in Gram-positive bacteria and from lipopolysaccharides and phospholipids in Gram-negative bacteria, as well as from the cytoplasmic membrane. Therefore, electrostatic attraction between the surface and bacteria is a key mechanism of contact-based antimicrobial activity (Santoro & Izzo, 2024). In addition to antibacterial effects, QACs are also active against enveloped viruses, where they destabilize the lipid membrane. Cationic QAC, such as benzalkonium chlorides (BAC), can interact with the viral envelope, affecting its stability and fluidity. These changes may disrupt homeostasis, alter pH in endocytic and lysosomal vesicles, and ultimately lead to leakage, lysis, and viral inactivation (Mohapatra et al., 2023).

4. Chitosan

Although synthetic polymers offer advantages such as strength and durability, their major drawback is their non-biodegradability, which leads to long-term environmental persistence and pollution. For this reason, increasing attention has been directed toward natural, biodegradable materials. Among these, biopolymers have gained considerable interest due to their environmental compatibility and wide range of applications, including medicine, cosmetics, moisture absorption, and tissue regeneration. Chitosan is one of the most

extensively studied natural biopolymers. It is derived from the shells of marine organisms such as shrimp and crabs and possesses several advantageous properties, including biodegradability, biocompatibility, non-toxicity, non-allergenicity, affordability, and various therapeutic effects. These include antimicrobial, antitumor, antioxidant, and immunostimulatory activities. In addition, its structure can be readily modified, enabling the development of derivatives for diverse biomedical applications (Confederat et al., 2021). Chitosan exhibits antimicrobial activity against a wide range of bacteria, including both Gram-positive species (e.g., *Staphylococcus aureus*) and Gram-negative species (e.g., *Escherichia coli*), as well as other pathogens such as *Salmonella*, *Listeria*, and *Pseudomonas* (Ngo et al., 2015). However, one limitation of chitosan is its poor solubility in water, which is attributed to its rigid and crystalline structure (Xie et al., 2007). Nevertheless, the presence of amino groups allows for chemical modification, which can improve solubility and enhance antimicrobial efficacy. Modified forms of chitosan have been developed that exhibit improved solubility and higher antibacterial activity, even at neutral pH, which is particularly important for biomedical applications (Confederat et al., 2021).

4.1. Broad-spectrum antimicrobial activity of chitosan

The antibacterial activity of chitosan and its derivatives is primarily driven by electrostatic interactions. Chitosan carries a positive charge, whereas bacterial cell surfaces are negatively charged. This interaction leads to disruption of the bacterial envelope—affecting the cell wall in Gram-positive bacteria and the outer membrane in Gram-negative bacteria. As a result, membrane permeability increases, allowing essential intracellular components such as enzymes, ions, and nucleotides to leak out, ultimately leading to cell death (Confederat et al., 2021). Numerous studies over the past two decades have demonstrated that the antibacterial effectiveness of chitosan depends on multiple factors. These include intrinsic properties such as molecular weight, degree of purification, and concentration, as well as external factors such as bacterial type and age, with younger cells generally being more susceptible. Environmental conditions, including pH, temperature, and the presence of metal ions (e.g., calcium or magnesium), also significantly influence its activity (Kong et al., 2010).

In addition to antibacterial effects, chitosan also exhibits antiviral properties, which are particularly relevant in the context of difficult-to-treat viral infections such as HIV and hepatitis C. Low-molecular-weight chitosan oligosaccharides have been shown to inhibit HIV replication at non-toxic concentrations by preventing syncytium formation, reducing viral antigen (p24) production, and blocking viral entry and fusion with host cell membranes. These findings suggest potential for the development of novel antiviral therapies (Artan et al., 2010). Furthermore, chitosan-conjugated nanoparticles have demonstrated the ability to target HIV, prolong drug activity, and reduce side effects, offering potential for improved antiretroviral treatments (Ngo et al., 2015). Studies using animal models have also shown that chitosan can be effective in preventing infection with influenza A (H7N9). Rather than acting directly on the virus, chitosan appears to stimulate the host immune response, enabling a more effective defense during the early stages of infection(Zheng et al., 2016).

Chitosan also demonstrates antifungal activity, although the underlying mechanisms are not yet fully understood. It has been shown to be effective against various fungi, particularly *Candida* species, including drug-resistant strains such as those resistant to fluconazole. Its activity depends on factors such as molecular weight, degree of deacetylation, concentration, and pH, with optimal effects observed at lower pH and concentrations between 1–5%. Chitosan disrupts fungal cell membranes, increases membrane permeability, causes the loss of essential ions (e.g., potassium), and inhibits respiration and metabolism. Some studies have also reported synergistic effects when chitosan is combined with antifungal drugs such as fluconazole, although this effect depends on the specific type of chitosan and drug used (Confederat et al., 2021).

5. Graphene

Graphene is one of the most promising materials in modern science. Due to its exceptional mechanical strength, large surface area, excellent thermal and electrical properties, and capacity for chemical modification, it has attracted significant attention, particularly in biomedical applications. Research over recent decades has demonstrated that graphene is not only versatile and robust but also biocompatible, antibacterial, and corrosion-resistant,

which is particularly important for medical implants and dental materials. Despite extensive research, the application of graphene coatings in dentistry and implantable medical devices remains limited, indicating substantial potential for future development (Srimaneepong et al., 2022). Graphene-based materials exhibit strong antibacterial properties, effectively inhibiting bacterial growth and proliferation. When applied as coatings on titanium, a commonly used implant material, they can significantly reduce biofilm formation and enhance resistance to microbial infections. In one study, graphene coatings reduced biofilm formation by bacteria such as *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Streptococcus mutans*, as well as fungi such as *Candida albicans*, by approximately 50% after only two applications. This effect has been attributed to the hydrophobic nature of graphene, which limits bacterial adhesion to surfaces (Agarwalla et al., 2019). Graphene derivatives, particularly graphene oxide (GO) and reduced graphene oxide (rGO), are highly effective antimicrobial materials that act against a wide range of bacteria and fungi. Their activity is mainly associated with the induction of oxidative stress and damage to the cell membrane. Although their effectiveness depends on material properties and the type of microorganism, they generally exhibit strong and broad-spectrum antimicrobial activity (Pandit et al., 2021). Graphene and its derivatives are typically more effective against Gram-positive bacteria, which is related to differences in cell wall structure. Gram-negative bacteria possess an additional outer membrane rich in lipopolysaccharides, providing increased protection against external stress. Furthermore, graphene-based materials show significant potential against antibiotic-resistant bacteria, making them promising candidates for future medical applications (Mohammed et al., 2020).

5.1. Antimicrobial Activity of graphene

The antimicrobial activity of graphene and its derivatives can be explained by several interconnected mechanisms. A key factor is mechanical damage, as the sharp edges of graphene sheets can disrupt bacterial membranes, leading to loss of integrity and leakage of intracellular contents. Another important mechanism is the induction of oxidative stress, either through the generation of reactive oxygen species (ROS) or via ROS-independent pathways, which disrupt essential cellular functions and lead to cell death. In addition, graphene sheets can physically wrap around and trap bacterial cells, isolating them from their environment, limiting nutrient availability, and preventing proliferation. This trapping effect further enhances membrane damage and contributes to the overall antimicrobial activity (Mohammed et al., 2020; Pandit et al., 2021).

6. Conclusions

Quaternary ammonium compounds are highly effective antimicrobial agents, making them a popular choice in many applications. However, their synthetic nature may pose environmental concerns, and the potential development of microbial resistance limits their long-term use. In contrast, chitosan, as a natural material, offers important advantages in terms of biocompatibility and environmental sustainability. Its antiviral properties further enhance its potential for applications in medicine and pharmaceuticals. Nevertheless, its limited solubility represents a practical challenge, often requiring modification or combination with other materials to achieve optimal performance.

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