

1. Introduction

1.1. Antimicrobial resistance

Antimicrobial resistance (AMR) has become one of the most pressing public health challenges of the 21st century, threatening the effective prevention and treatment of a wide array of infections caused by bacteria, viruses, fungi, and parasites [1, 2]. Once hailed as miracle drugs, antimicrobials—including antibiotics, antivirals, antifungals, and antiparasitics—have saved countless lives since their introduction. However, the widespread and often inappropriate use of these agents in human medicine, agriculture, and animal husbandry has accelerated the evolution and dissemination of resistance among pathogenic microorganisms [3]. As a result, infections that were once easily treatable are increasingly becoming difficult, and in some cases, impossible to cure, leading to higher morbidity, mortality, and healthcare costs.

AMR occurs when microorganisms develop mechanisms that enable them to withstand the effects of antimicrobials designed to eliminate them. These mechanisms include limiting drug uptake, modifying drug targets, inactivating drugs, and actively expelling drugs from the cell [4]. Resistance can arise through spontaneous genetic mutations or via horizontal gene transfer, allowing resistance traits to spread rapidly within and between microbial populations. The phenomenon is not restricted to a single class of microbes; all major groups—including bacteria (antibiotic resistance), viruses (antiviral resistance), fungi (antifungal resistance), and parasites (antiparasitic resistance)—are affected [2].

The scale of the problem is alarming: in 2019 alone, an estimated 1.27 million deaths were directly attributable to antimicrobial-resistant infections, with nearly 5 million deaths associated in some way with AMR. Without urgent and coordinated global action, projections suggest that AMR could cause up to 10 million deaths annually by 2050,

surpassing the mortality rates of many major diseases today. The economic impact is equally severe, with prolonged hospital stays, the need for more expensive therapies, and increased societal costs due to lost productivity [5].

Key drivers of AMR include the misuse and overuse of antimicrobials in both healthcare and agriculture, poor infection prevention and control practices, inadequate sanitation, and insufficient surveillance and regulatory frameworks. The emergence of multidrug-resistant (MDR) organisms, often termed "superbugs," further complicates treatment options and increases the risk of untreatable infections. Compounding the crisis is the stagnation in the development of new antimicrobials, which has not kept pace with the rapid evolution of resistance [6].

In response, the World Health Organization and other international bodies have called for comprehensive strategies encompassing improved surveillance, stewardship of existing antimicrobials, investment in research and development of new drugs, vaccines, and diagnostics, and the implementation of robust infection control measures. Without such concerted efforts, the world risks entering a "post-antibiotic era," where even minor infections could once again prove fatal [7].

This thesis aims to provide an in-depth analysis of the mechanisms underlying antimicrobial resistance, the factors driving its emergence and spread, and the multifaceted strategies required to combat this escalating global threat.

1.1.1. AMR threaten global health and future treatments

AMR poses an escalating threat to global health and medical advancements through multiple interconnected mechanisms. Below is a structured analysis of its impacts and implications [8]:

1.1.1.1. Immediate health threats

1. Rising mortality and treatment failures

AMR caused 1.27 million direct deaths in 2019, with nearly 5 million deaths linked to resistant infections. Common pathogens like *E. coli*. and *Klebsiella pneumoniae* now show 35–42% resistance to first-line antibiotics, complicating treatments for urinary tract infections, sepsis, and pneumonia.

2. Compromised medical procedures

Routine surgeries (e.g., cesarean sections), cancer chemotherapy, and organ transplants rely on effective antimicrobials to prevent infections. AMR increases the likelihood of post-procedure complications, turning minor interventions into life-threatening events.

3. Escalating antimicrobial inefficacy

Over 70% of pathogenic bacteria resist at least one antibiotic. For example, fluoroquinolone resistance in *E. coli*. exceeds 20% globally, while methicillin-resistant *Staphylococcus aureus* (MRSA) complicates skin and soft-tissue infections.

1.1.1.2. Long-term risks to future treatments

1. Stagnant drug development pipeline

Only 11 new antibiotics entered clinical trials between 2017 and 2021, with most representing modifications of existing classes. Pharmaceutical companies often deprioritize antibiotic R&D due to low profitability, risking a "post-antibiotic era".

2. Emergence of pan-resistant pathogens

Carbapenem-resistant *Acinetobacter baumannii* and extensively drug-resistant tuberculosis highlight pathogens evolving beyond available treatments. Projections suggest double the resistance rates to last-resort drugs by 2035 compared to 2005.

3. Environmental and agricultural drivers

Pollution from pharmaceuticals, agriculture, and healthcare waste accelerates resistance gene spread. For example, 70% of antibiotics are used in livestock, fostering resistant strains that transfer to humans.

1.1.2. Strategies to mitigate AMR

- **Preventive measures:** Strengthen sanitation, vaccination programs, and infection control in healthcare settings.
- **Stewardship:** Reduce antibiotic misuse in humans (e.g., 72% of COVID-19 patients received unnecessary antibiotics) and agriculture.
- **Innovation:** Develop immuno-antibiotics targeting bacterial metabolic pathways and inhibit resistance mechanisms like SOS response and hydrogen sulfide production.

Without coordinated global action, AMR could claim 10 million lives annually by 2050, surpassing cancer mortality and reversing decades of medical progress. Addressing this crisis requires urgent investment in surveillance, equitable drug access, and novel therapeutic strategies [9].

1.1.3. AMR in key pathogens: mechanisms and implications

1. ***Escherichia coli*:** A model of horizontal gene transfer and multidrug resistance

Resistance Mechanisms

- Extended-Spectrum β -Lactamases (ESBLs): Confers resistance to cephalosporins via enzymatic hydrolysis.
- Carbapenemases: Target carbapenems (e.g., KPC, NDM enzymes), often plasmid-borne.
- Plasmid-Mediated Quinolone resistance (PMQR): Alterations in target enzymes (e.g., gyrA, parC) or efflux pumps.

- Polymyxin resistance (*mcr* genes): Encodes phosphoethanolamine transferases modifying lipid A.

Clinical impact

- Cross-Species Spread: Colistin resistance in veterinary *E. coli* strains highlights zoonotic risks.
- MDR Prevalence: ~26% of neonatal isolates exhibit resistance to ≥ 3 antibiotic classes [10].

2. *Pseudomonas aeruginosa*: Intrinsic and adaptive resistance

Resistance Mechanisms

- Intrinsic Resistance: Low membrane permeability, Mex-type efflux pumps, and AmpC β -lactamase.
- Acquired Resistance:
 - Aminoglycoside resistance: 16S rRNA methylases (e.g., *armA*).
- Adaptive Strategies: Biofilm formation (alginate production) and persister cells.

Clinical impact

- MDR Strains: 18.7% of isolates are carbapenem-resistant in Europe; pan-drug resistance (PDR) reported.
- Chronic Infections: Biofilms in cystic fibrosis (CF) lungs evade immune responses and antibiotics [10].

3. *Staphylococcus aureus*: Methicillin resistance and beyond

Resistance mechanisms

- Methicillin resistance (MRSA):
 - *mecA/mecC* genes encode PBP2a/PBP2c, bypassing β -lactam inhibition.
 - SCC*mec* element carries *mecA* and auxiliary *fem* genes (e.g., *femA*, *femB*).

- Daptomycin resistance: Mutations in *mprF* (lysylphosphatidylglycerol synthesis) or *yycFG* (cell membrane homeostasis).
- Macrolide/Lincosamide resistance: *erm* and *msrA* genes alter ribosomal targets.

Clinical impact

- High resistance rates: 82–83% resistance to erythromycin and clindamycin.
- Emerging threats: Phage-resistant MRSA strains reduce bacteriophage therapy efficacy [11].

4. *Candida albicans*: Azole resistance and biofilm persistence

Resistance mechanisms

- Azole resistance:
 - ERG11 mutations (e.g., Y132F, K143R) reduce ergosterol biosynthesis.
 - Upregulation of ABC transporters (CDR1, CDR2) and MFS pumps (MDR1) via TAC1/MRR1 activation.
- Echinocandin resistance: FKS1/FKS2 mutations alter β -1,3-glucan synthase.
- Biofilm formation: Exopolysaccharides sequester drugs, reducing efficacy.

Clinical impact

- Multidrug resistance: *C. glabrata* and *C. auris* exhibit intrinsic/flucytosine resistance.
- High mortality: Bloodstream infections with MDR strains correlate with prolonged antifungal exposure [12].

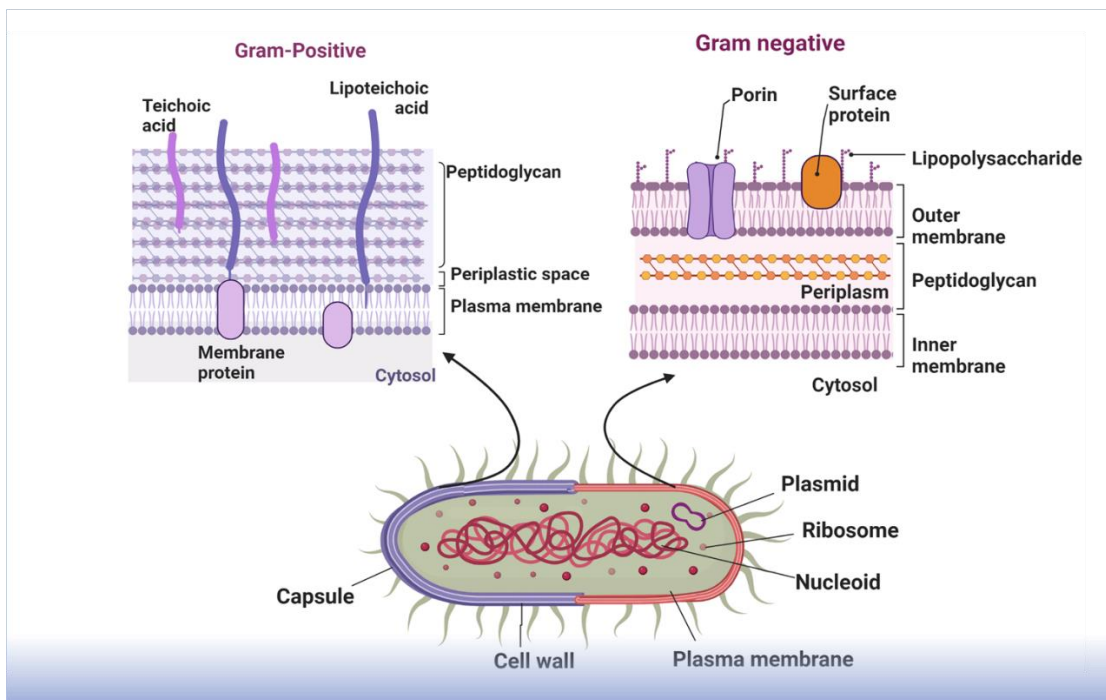


Figure 1.1. Antimicrobial mechanism of gram-positive and gram-negative bacteria

1.2. Nanoparticles as an alternative treatment to AMR

In response to this urgent challenge, the scientific community has intensified efforts to discover alternative antimicrobial strategies that can bypass traditional resistance mechanisms. Among the most promising innovations are metallic and bimetallic nanoparticles, which consist of single or two distinct metal elements integrated at the nanoscale [13]. These nanomaterials have demonstrated superior antimicrobial properties compared to their monometallic counterparts, owing to synergistic effects that enhance their bactericidal and fungicidal activities. The unique physicochemical properties of bimetallic nanoparticles such as increased surface area, enhanced reactivity, and the ability to generate reactive oxygen species (ROS)—enable them to disrupt microbial cell membranes, interfere with metabolic processes, and inhibit biofilm formation [14, 15].

1.2.1. Metallic nanoparticles

Metallic nanoparticles have emerged as a promising alternative in the battle against AMR due to their unique physicochemical properties and multifaceted mechanisms of action [16]. Unlike traditional antibiotics, which typically target specific bacterial pathways or structures, metallic nanoparticles exert their antimicrobial effects through multiple, often simultaneous, mechanisms [17]. These include direct disruption of microbial cell membranes, generation of ROS, release of toxic metal ions, and interference with intracellular processes such as enzyme activity and DNA replication. Such broad-spectrum activity reduces the likelihood that bacteria will rapidly develop resistance to metallic nanoparticles, as adaptation would require simultaneous mutations across multiple cellular targets [18].

Various metallic nanoparticles—including silver (Ag-NPs), gold (Au-NPs), zinc oxide (ZnO-NPs), copper (Cu-NPs), and platinum (Pt-NPs)—have demonstrated potent activity against a wide range of MDR bacteria and fungi [19, 20]. For instance, silver nanoparticles have shown remarkable efficacy in disrupting bacterial membranes and biofilms, while gold and zinc oxide nanoparticles have been successfully used to enhance the antimicrobial activity of existing antibiotics, often displaying synergistic effects. These nanoparticles can also be functionalized or combined with antibiotics and natural products, further increasing their potency and expanding their spectrum of action [18].

The physicochemical properties of metallic nanoparticles such as size, shape, surface charge, and functionalization significantly influence their antimicrobial efficacy. Smaller nanoparticles, particularly those below 10 nm, exhibit greater surface area and enhanced interaction with microbial cells, leading to increased toxicity and biofilm penetration. Surface charge is also critical, with positively charged nanoparticles generally

showing higher affinity for negatively charged bacterial membranes, resulting in more effective membrane disruption [19].

Despite these promising attributes, several challenges must be addressed before metallic nanoparticles can be widely adopted in clinical practice. These include concerns about cytotoxicity to human cells, environmental impact, and the potential for bacteria to develop resistance to nanoparticles if misused. Recent evidence suggests that, under continuous sublethal exposure, bacteria may evolve resistance mechanisms against certain nanoparticles, underscoring the need for careful management and further research into their safe and effective application [21].

Ag-NPs are among the most commonly investigated for their potential antimicrobial applications (Eg.- Acticoat©, Aquacel Ag+, Silvasorb™) [22].

1.2.2. Bimetallic nanoparticles

Recent studies have shown that bimetallic nanoparticles, including combinations like silver-iron (Ag–Fe), gold-silver (Au–Ag), and silver-copper (Ag–Cu), exhibit potent activity against a broad spectrum of clinically relevant, drug-resistant pathogens [23]. For example, Ag–Fe bimetallic nanoparticles synthesized via green chemistry approaches have demonstrated synergistic antimicrobial effects against both Gram-positive and Gram-negative bacteria, as well as yeast, while minimizing environmental hazards associated with nanoparticle production [24]. Similarly, Au–Ag and Ag–Cu bimetallic nanoparticles have been found to be highly effective against MDR strains, with some formulations outperforming conventional antibiotics in both *in vitro* and *in vivo* models. [23]

The development of bimetallic nanoparticles as antimicrobial agents represents a paradigm shift in the fight against AMR. These nanomaterials not only offer a substitute for failing antibiotics but also provide new avenues for infection control, wound healing, and the prevention of biofilm-associated infections on medical devices [24].

Bimetallic nanoparticles exhibit distinctive and impactful properties that underscore their scientific and technological importance in domains including:

1. **Synergistic antimicrobial potency:** The galvanic couple enhances ROS generation and releases Ag^+ ions more steadily, giving broad-spectrum activity [25].
2. **Lower cytotoxicity & better biocompatibility than silver-only systems:** Incorporating an inert Au domain reduces silver dissolution bursts, decreasing oxidative stress on keratinocytes/fibroblasts while maintaining microbicidal activity—an advantage for chronic-wound dressings.
3. **Accelerated wound closure:** Animal models show Au-Ag-NPs-loaded carrier shorten re-epithelialization time, stimulate collagen deposition, and minimize scarring owing to simultaneous antibacterial, anti-inflammatory, and pro-angiogenic effects [26].
4. **Multimodal mechanisms:** Membrane disruption by Ag^+ , ROS-mediated protein/DNA damage collectively kill microbes and improve blood microcirculation in the wound bed [25].

However, challenges remain regarding their large-scale synthesis, biocompatibility, and potential cytotoxicity, which must be addressed through continued research and clinical evaluation.

1.3. Chitosan

Chitosan, a positively charged biopolymer with a versatile structure and reactive functional groups, is synthesized via chitin deacetylation which ranks second among the most prevalent natural polysaccharides after cellulose [27]. Chitin occurs in various biological sources, including green algae, fungal cell walls, crustacean exoskeletons (e.g., shrimp, lobster, crab), and the cuticles of arachnids and insects, where it provides mechanical strength and structural support [28]. The molecular framework of chitosan consists of β -(1-

4)-linked 2-amino-2-deoxy- β -D-glucose (D-glucosamine) and N-acetyl-D-glucosamine residues. The percentage of β -1,4-D-glucosamine units in the structure of chitosan represents the degree of deacetylation (DD), a key characteristic that influences its properties. This value varies depending on the source and the method of preparation, with molecular weights ranging from 300 kDa to over 1000 kDa and DD values spanning from 30% to 95% [29]. Chitosan could be classified into low, medium, or high molecular weight categories based on its DD value. Low-molecular-weight chitosan has shown effectiveness in improving solubility, reducing the size of particles, and increasing the stability of nanocrystal formulations. Chitosan is typically insoluble in water with a pH higher than 7 where it is present in its crystalline form. However, in more acidic conditions (pH < 6.0), the amino groups on glucosamine become protonated, enhancing the molecule's solubility [30].

Its desirable properties include biocompatibility, biodegradability, hydrophilicity, low toxicity, high bioavailability, and ease of chemical modification [31]. Additionally, it exhibits selective water permeability, chemical stability, and the potential to form multifarious systems such as nanoparticles, films, microparticles, and beads, while demonstrating strong binding affinity for metals, proteins, and dyes. Upon degradation in the human body, chitosan is metabolized into amino sugars that are readily absorbed [32].

1.3.1. N,N,N-Trimethyl chitosan

The escalating crisis of AMR has catalyzed the search for innovative alternatives to conventional antibiotics. Among the most promising candidates are chitosan derivatives, particularly N,N,N-trimethyl chitosan (TMC), and their nanoparticle formulations. TMC, a quaternized derivative of chitosan, exhibits superior solubility and antimicrobial efficacy compared to its parent compound, especially under neutral and alkaline conditions where native chitosan's activity is limited [33, 34]. The development of TMC-based nanoparticles

further amplifies these properties, offering enhanced bioactivity, stability, and potential for clinical translation in combating resistant pathogens [35].

1.3.1.1. Trimethyl chitosan: structure and advantages

Trimethyl chitosan is synthesized by introducing methyl groups onto the amino sites of chitosan (**figure 1.2**), resulting in a permanently cationic polymer across a broad pH range. This modification not only improves water solubility but also increases the density of positive charges, thereby strengthening electrostatic interactions with negatively charged microbial surfaces and biofilm matrices. TMC's enhanced mucoadhesive and bioadhesive properties also make it an attractive candidate for drug delivery and antimicrobial applications [36, 37].

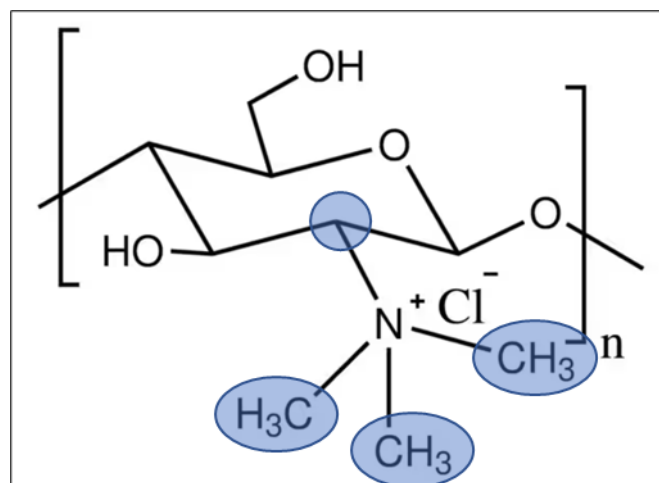


Figure 1.2. Structure of N,N,N-Trimethyl chitosan

1.3.1.2. TMC-based nanoparticles: synthesis and properties

The formulation of TMC into nanoparticles (TMC-NPs) leverages nanotechnology to further boost its antimicrobial potential. TMC-NPs typically exhibit a median size around 100 nm, a positive surface charge, and high colloidal stability, even in complex biological environments [38]. Nanoparticle formation can also mitigate the cytotoxicity sometimes observed with highly methylated TMC, improving safety profiles for pharmaceutical use [39].

1.3.1.3. Antimicrobial uses of TMC and TMC-based nanoparticles

TMC and its nanoparticles demonstrate broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi. Notably, TMC outperforms native chitosan in inhibiting pathogens such as *S. aureus*, *E. coli.*, and *P. aeruginosa*, including drug-resistant and biofilm-forming strains [39]. TMC-NPs have also shown efficacy in reducing biofilm development by up to 50% at concentrations between 80 and 160 mg/L, a significant advance given the role of biofilms in chronic and device-associated infections [40, 41].

1.3.1.4. Applications

- **Medical device coatings:** Preventing bacterial colonization and biofilm formation.
- **Wound dressings:** Promoting healing while suppressing infection.
- **Drug delivery:** Enhancing the delivery and retention of antibiotics or other therapeutics at infection sites.
- **Food preservation and personal care:** Reducing pathogen contamination.

1.3.1.5. Mechanisms of antimicrobial action

The antimicrobial efficacy of TMC and its nanoparticles is attributed to several interrelated mechanisms:

- **Electrostatic disruption:** The high density of positive charges on TMC enables strong electrostatic interaction with the negatively charged microbial cell wall and membrane components, such as lipopolysaccharides and teichoic acids. This interaction increases membrane permeability, leading to leakage of intracellular contents and cell death [41].
- **Biofilm inhibition:** TMC disrupts the extracellular polymeric substances (EPS) within biofilms, impeding their structural integrity and facilitating deeper penetration of the antimicrobial agent. This antibiofilm activity is particularly

pronounced in TMC-NPs, which can reduce biofilm mass and viability by direct interaction with the biofilm matrix [42].

- **Intracellular interference:** TMC can enter microbial cells and bind to DNA, thereby inhibiting transcription and protein synthesis, further suppressing microbial growth [43].
- **Modulation of virulence factors:** TMC-NPs have been shown to affect bacterial virulence traits such as motility, protease activity, and hemolysin production, although these effects are likely due to non-specific surface interactions rather than direct interference with quorum sensing [44].
- **Tight junction modulation:** In drug delivery applications, TMC-NPs can transiently open epithelial tight junctions via the C-Jun N-terminal kinase-dependent pathway, enhancing paracellular transport without permanent tissue damage [43].

1.3.1.6. Safety and biocompatibility

While TMC's cytotoxicity can be influenced by its degree of methylation and molecular weight, nanoparticle formulation has been shown to reduce cytotoxic effects, enhancing compatibility with human cells and broadening its therapeutic window. This positions TMC-NPs as safer alternatives for clinical and pharmaceutical applications [40, 41].

1.3.2. Carboxy methyl chitosan

The global escalation of AMR has intensified the search for innovative, sustainable, and effective alternatives to conventional antibiotics. Among the most promising candidates are chitosan derivatives particularly carboxymethyl chitosan (CMC) and their bimetallic nanoparticle composites. These materials not only leverage the inherent antimicrobial properties of chitosan but also harness the enhanced bioactivity and multifunctionality of

bimetallic nanoparticles, offering new avenues for combatting resistant pathogens and reducing reliance on traditional drugs [45, 46].

1.3.2.1. Carboxymethyl chitosan: structure and advantages

Carboxymethyl chitosan is a water-soluble derivative of chitosan, produced by introducing carboxymethyl groups onto the chitosan backbone [46]. This modification significantly improves its solubility and biocompatibility, allowing for broader biomedical applications compared to native chitosan. CMC retains the cationic nature of chitosan, enabling strong interactions with microbial cell surfaces, while its carboxyl groups provide additional sites for metal ion binding and nanoparticle stabilization [45, 47].

1.3.2.2. CMC-based bimetallic nanoparticles: synthesis and properties

CMC serves as an ideal matrix for the synthesis and stabilization of bimetallic nanoparticles, such as silver-platinum (AgPt), silver-gold (Ag-Au), or silver-copper (Ag-Cu) systems. The carboxyl and amino groups of CMC facilitate the reduction of metal ions and act as capping agents, controlling nanoparticle size, shape, and dispersion. The hybridization of CMC with bimetallic nanoparticles or even metal-organic frameworks (MOFs) creates "metal ion warehouses"—structures capable of rapid sterilization and prolonged antimicrobial activity due to controlled metal ion release [45].

Bimetallic nanoparticles exhibit synergistic effects, often outperforming their monometallic counterparts in antimicrobial activity. For example, AgPt nanoparticles embedded in chitosan-based matrices have demonstrated superior efficacy against bacteria, yeast, and filamentous fungi compared to standard antibiotics. The combination of two metals can enhance ROS generation, disrupt multiple microbial targets, and reduce the likelihood of resistance development.

1.3.2.3. Antimicrobial uses and mechanism of action

CMC and its bimetallic nanoparticle composites display broad-spectrum antimicrobial activity against a range of pathogenic microorganisms, including *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* [47]. The mechanism of action of CMC are:

- **Membrane disruption:** CMC-based nanoparticles interact electrostatically with negatively charged microbial membranes, leading to increased permeability, leakage of cellular contents, and cell death [47].
- **Metal ion release and ROS generation:** Bimetallic nanoparticles release metal ions (e.g., Ag^+ , Pt^{2+}) that interact with thiol groups in proteins and enzymes, disrupting cellular metabolism. Simultaneously, they catalyze the production of ROS, which damage DNA, proteins, and lipids, amplifying the antimicrobial effect [48].
- **Biofilm inhibition:** The nanoparticles can penetrate and disrupt biofilm matrices, preventing the establishment and persistence of chronic infections [47, 48].
- **Synergistic effects:** The presence of two metals in bimetallic nanoparticles creates multiple modes of action, making it more difficult for microbes to develop resistance [48].

Studies have shown that CMC-embedded silver nanoparticles, for example, exhibit excellent antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi, outperforming either CMC or silver nanoparticles alone. The hybrid materials can be fabricated into films, coatings, or wound dressings, providing controlled and sustained antimicrobial action suitable for medical and environmental applications [49].

1.4. Nanofibers

Nanofibers, defined as fibers with diameters typically ranging from 1 nanometer to 1 micrometer, represent a class of advanced materials that have garnered significant attention

across scientific and industrial domains due to their unique structural and functional properties [50]. Distinguished by their exceptionally high surface area-to-volume ratio, high porosity, and tunable mechanical strength, nanofibers can be fabricated from a wide array of natural and synthetic polymers, including but not limited to chitosan, cellulose, poly(lactic acid), polycaprolactone, and polyvinyl alcohol [51, 52]. This versatility in composition and structure has enabled nanofibers to find applications in diverse fields such as biomedicine, environmental remediation, filtration, energy storage, and advanced textiles.

1.4.1. Synthesis and structural features of nanofibers

The fabrication of nanofibers has evolved through several sophisticated techniques, with electrospinning emerging as the most prevalent method due to its simplicity, scalability, and ability to produce continuous fibers with controlled diameters and morphologies. Other notable synthesis approaches include self-assembly, template synthesis, and phase separation, each offering specific advantages in terms of fiber alignment, dimension control, and compositional diversity [50]. The electrospinning process, in particular, enables the fine-tuning of fiber properties by adjusting parameters such as polymer concentration, applied voltage, and flow rate, resulting in nanofibers with tailored surface characteristics and porosity [51].

The defining features of nanofibers—namely, their nanoscale diameter, high surface area, interconnected pore structure, and mechanical flexibility—confer a range of functional benefits. For instance, the small fiber diameter and high porosity facilitate efficient mass transport, selective adsorption, and enhanced interaction with biological or chemical species, making nanofibers ideal for applications in filtration, catalysis, and biomedical engineering [50-52].

1.4.2. Functional applications of nanofibers

Nanofibers have revolutionized several technological sectors:

- **Biomedical field:** In tissue engineering, nanofibers serve as scaffolds that mimic the extracellular matrix, supporting cell attachment, proliferation, and differentiation. Their use in wound dressings is particularly notable, as nanofiber mats provide a moist healing environment, absorb exudates, and offer protection against microbial invasion. Drug delivery systems based on nanofibers allow for controlled and targeted release of therapeutics, improving treatment efficacy and reducing side effects [52].
- **Filtration and environmental remediation:** The high surface area and tunable pore size of nanofiber membranes make them highly effective for air and water filtration, capable of removing submicron particles, pathogens, and volatile organic compounds with high efficiency. Nanofiber-based filters are also used in personal protective equipment, such as face masks, due to their ability to block bacteria and viruses while maintaining breathability [53].
- **Sensors and electronics:** Nanofibers are integrated into chemical and biosensors, leveraging their sensitivity and rapid response to detect environmental pollutants, biomolecules, or gases. Their incorporation into flexible electronics and energy storage devices, such as batteries and supercapacitors, enhances performance due to their conductive and high-capacitance properties [54].
- **Textiles and composites:** In advanced textiles, nanofibers contribute to improved breathability, moisture management, and antimicrobial properties, while in composite materials, they enhance mechanical strength and functional performance [55].

1.4.3. Antimicrobial and bactericidal mechanisms of nanofibers

A particularly significant application of nanofibers is in the development of antimicrobial materials [56]. Electrospun nanofibers can be engineered to exhibit strong biocidal activity by incorporating antimicrobial agents—such as antibiotics, metal nanoparticles, or plant-derived compounds—or by utilizing inherently antimicrobial polymers like chitosan [52]. The small diameter and high surface area of nanofibers enable efficient encapsulation and sustained release of these agents, while the porous structure promotes interaction with microbial cells [50].

Mechanistically, nanofibers exert their antimicrobial effects through several pathways:

- **Physical disruption:** The close contact between nanofibers and microbial membranes can lead to membrane destabilization and cell lysis.
- **Chemical interactions:** Encapsulated or surface-bound antimicrobial agents are released in a controlled manner, inducing oxidative stress, disrupting metabolic pathways, or interfering with cell wall synthesis in bacteria and fungi [56].
- **Biofilm inhibition:** Nanofibers can prevent the formation of biofilms or penetrate existing biofilms, thereby enhancing the efficacy of antimicrobial treatments [57].

1.4.4. Nanofibers loaded with nanoparticles

In certain biomedical applications, such as tissue regeneration, a significant challenge and critical concern is achieving a continuous and controlled release of active compounds or growth factors. Nanoparticles loaded nanofibers composites have received much attention as a potential solution to this problem. Thus, nanofibers are considered a promising material for wound dressing, with potential applications in the treatment of diverse injuries and infections [58-60]. Recently, nanofiber dressings consisting of nanoparticles have gained popularity due to their ability to fulfill the complicated needs of the biological environment

at the site of bacterial infection, which standard nanofiber dressings are unable to fulfill [61].

1.4.5. Nanofibers as wound dressing for tissue engineering

The management of wounds, particularly those that are chronic or complex, remains a significant challenge in clinical practice and tissue engineering. Traditional wound dressings, such as gauze and cotton pads, primarily serve as physical barriers, providing basic protection but often lacking the ability to actively support the biological processes essential for effective tissue repair [61]. As a result, there has been a growing demand for advanced wound care materials that not only protect the wound site but also facilitate the intricate cascade of healing events, including hemostasis, inflammation, proliferation, and remodeling [58, 59].

Nanofiber-based wound dressings have emerged at the forefront of this innovation, offering a unique combination of structural and functional advantages that closely mimic the extracellular matrix (ECM) of native tissues. Nanofibers, typically produced through electrospinning and related techniques, are characterized by their ultrafine diameters, high surface area-to-volume ratios, and interconnected porous networks. These features provide an ideal environment for cell adhesion, proliferation, and migration—key processes in tissue regeneration and wound healing [60].

One of the most compelling attributes of nanofibrous dressings is their ability to be engineered from a wide variety of natural and synthetic polymers, or their blends, allowing for the customization of mechanical strength, degradation rates, and biological interactions. For example, incorporating biopolymers such as chitosan, collagen, or gelatin can enhance biocompatibility and bioactivity, while synthetic polymers like polycaprolactone or polylactic acid can impart structural stability and controlled degradation. This versatility

enables the fabrication of scaffolds tailored to specific wound types and healing requirements [61].

Beyond their structural role, nanofiber dressings can serve as carriers for a range of bioactive agents, including antimicrobial compounds, growth factors, and anti-inflammatory drugs. The high surface area and porous architecture of nanofibers facilitate the encapsulation and sustained release of these agents directly at the wound site, reducing the risk of infection, modulating inflammation, and accelerating tissue repair [62]. This multifunctionality is particularly valuable in the context of antimicrobial resistance, where localized delivery of therapeutics can enhance efficacy while minimizing systemic exposure and side effects.

Furthermore, nanofibers can be designed to mimic the physical and biochemical cues of the ECM, guiding cellular behavior and supporting the regeneration of complex tissues [63]. Their ability to modulate cell attachment, migration, and differentiation makes them highly suitable for applications in tissue engineering, where the goal is not only to heal wounds but also to restore the structure and function of damaged tissues.

Recent advances in nanofiber technology have led to the development of smart and multifunctional wound dressings, incorporating features such as temperature sensitivity, moisture regulation, and responsive drug release. These next-generation materials are poised to address the challenges of chronic wounds, diabetic ulcers, and burns, offering improved healing outcomes and enhanced patient comfort (Eg. - Mimetix©, Nanoderm™, Spincare©) [64, 65].