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A One Health Nanotechnologies Approach to Address Antimicrobial Resistance: State of the Art and Strategic Outlook

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ABSTRACT

Antimicrobial resistance (AMR) has emerged as a critical global health threat, driven by the rapid evolution and dissemination of resistance mechanisms among pathogens, and exacerbated by the interconnectedness of human, animal, and environmental health sectors. This review provides a comprehensive synthesis of the mechanisms underlying AMR including membrane permeability modification, efflux pumps, enzymatic inactivation, and target site modification while framing the crisis within the One Health perspective that emphasizes cross-sectoral collaboration and holistic strategies. The article systematically evaluates current approaches to combating AMR, such as novel drug discovery, combination therapies, bacteriophage-based interventions, antimicrobial adjuvants, and antimicrobial peptides, highlighting their respective strengths and limitations. The core of the review focuses on the advances in nanotechnology-based strategies, detailing the antimicrobial potential of diverse nanomaterials including chitosan, PLGA nanoparticles, liposomes, solid lipid nanoparticles, metal and metal ion nanoparticles (e.g., zinc oxide, silver, copper, gold, titanium, magnetic, cobalt), carbon dots, dendrimers, and hydrogels. Special attention is given to their mechanisms of action, efficacy against multidrug-resistant organisms, and applicability across human, veterinary, and environmental contexts. Moreover, the review addresses the limitations of nanotechnology-based approaches, such as nanoparticle cytotoxicity, the potential for nanoparticle-induced resistance, and the toxicological and ecological risks posed to One Health ecosystems. By critically appraising these challenges, the review identifies key research gaps and regulatory hurdles that must be overcome to enable the safe and effective clinical translation of nano-antimicrobials. The article concludes by outlining future prospects for the field, advocating for interdisciplinary research, responsible stewardship, and innovative policy frameworks to sustain the fight against AMR and protect global health.

Keywords: One Health; Antimicrobial Resistance; Nanotechnology Approaches; Nanoparticles; Antibiotics

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1. INTRODUCTION

Antimicrobials encompass a broad category of compounds that either inhibit the action or kill pathogenic microorganisms such as bacteria, fungi, protozoa, and viruses. These compounds include both organic and synthetic varieties. Antibiotics were first studied in the early 20th century, with notable discoveries like Salvarsan and Penicillin[1], and their use was popularised during the following decades. The introduction of antimicrobials into medical practices has reduced the mortality rate of multiple potentially lethal conditions, such as pneumonia[2] and sepsis[3,4]. They are also utilised for complex treatments, including organ transplants and surgeries[5]. Based on their mechanisms of action, antibiotic agents are classified into two categories: bactericidal agents, which kill bacterial cells, and bacteriostatic agents, which inhibit bacterial growth without directly causing cell death[6]. However, over time, new strains of bacteria have emerged that maintain their viability even when exposed to high antibiotic concentrations. Similar patterns have been observed in non-bacterial pathogens like *Plasmodium falciparum*, the causative pathogen for malaria[7]. This capability of a microorganism to resist the activity of antimicrobial agents at concentrations that would typically be inhibitory is termed antimicrobial resistance (AMR)[8]. AMR has been associated with the overprescription and improper usage of antibiotics, as prolonged exposure to antimicrobials stimulates the development of resistance against them[9]. However, research suggests that AMR is not just a modern phenomenon, with resistance genes found in permafrost samples ranging from 3,000 - 3,000,000 years old[10,11]. This suggests that resistance arises through intrinsic evolutionary processes in the bacteria. In 2017, the World Health Organization (WHO) classified numerous antibiotic-resistant bacteria as “priority pathogens”, posing a severe risk to modern healthcare[12]. Developing new treatments for these infections has become a primary concern, as conventional methods are unable to combat them effectively[13]. Nanomaterials are emerging as a potential solution due to their ability to overcome resistance mechanisms.

As stated, a significant factor contributing to the development of AMR is the inappropriate utilisation of antibiotics during medical treatment. During the administration of antibiotic drugs, it is necessary to ensure that appropriate dosage concentrations are used. However, studies have shown that less than 50% of patients receive adequate or appropriate dosages of antibiotics during treatment[14,15]. Furthermore, it has been found that antibiotics are sometimes prescribed for non-bacterial infections as well. For instance, a meta-analysis of COVID-19 infections found that despite a low apparent rate of bacterial co-infection (6.9%), antibiotics had been prescribed for

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71% of infections[16]. In many developing countries, antibiotics do not require prescriptions and are often readily available over the counter[17]. Such incidences create conditions in which bacteria exist in concentrations of antibiotics well below the Minimum Inhibitory Concentrations (MIC). Even at non-inhibitory concentrations, antibiotics still exert external stress on bacteria, prompting adaptation processes. This stress can lead to the development of antimicrobial-resistant genes through mutations. Bacteria possessing these genes may proliferate more rapidly than their non-resistant counterparts, gaining favour through natural selection. Moreover, these resistance genes can be transferred to other non-resistant bacteria *via* Horizontal Gene Transfer (HGT), facilitating the spread of resistance to new species[18].

The rise of AMR is not solely attributable to medical treatments. Many farmers routinely administer antimicrobials to livestock in pursuit of better health and increased yields. This practice remains widespread in numerous low- and middle-income nations with minimal regulatory oversight[19]. However, due to varying antibiotic dosages in livestock, environments with low antibiotic concentrations are frequently created, providing ample opportunities for resistance to develop. This provides numerous opportunities for resistance to be developed. Several studies have identified the existence of antimicrobial-resistant bacteria among farmers and those in close proximity, suggesting transmission from livestock[20]. Other studies show that over 90% of antimicrobials administered to livestock are excreted into the environmental microbiome, where resistance can again develop[21]. Collectively, these factors significantly contribute to the global escalation of AMR. In 2014, the WHO released a report describing AMR as "a problem so serious that it threatens the achievements of modern medicine"[22]. Antibiotics form a substantial part of healthcare, but the rise in resistance has contributed to a spike in severe infections globally. According to the US Centers for Disease Control, approximately 2.8 million infections involving antibiotic-resistant pathogens occurred in 2019, resulting in 35,000 deaths[23]. Another study indicated that globally, around 4.95 million deaths may be linked to antimicrobial-resistant infections[24]. This issue also poses substantial economic risks. Prolonged hospital stays, ineffective treatments, and the escalating costs of less commonly used antibiotics are driving up treatment expenses rapidly. A 2023 report by the Organization for Economic Co-operation and Development (OECD) estimated that AMR costs its member nations $28.9 billion[25]. In the absence of effective, permanent measures to combat this issue, it is feared that such costs will continue to increase drastically.

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In this review, we explore the ongoing fight against AMR, an escalating global threat. While traditional methods are becoming less effective, a new weapon is emerging on the horizon: nanotechnology. Furthermore, this review will explore the emerging role of nanoparticles (NPs) as a promising strategy to combat AMR pathogens, highlighting their various types, formulations, and proposed mechanisms of antimicrobial action. We will also elaborate on the synergistic effects observed when NPs are combined with conventional antibiotics, emphasising how such combinations enhance targeted delivery and promote effective bacterial cell disruption. This field holds immense potential to combat AMR through various strategies, offering hope for the future of medicine. Moreover, this review highlights recent advances in the design and application of NPs and nanocomposites (NCs) aimed at tackling antibiotic-resistant and biofilm-producing bacteria. As a rapidly evolving interdisciplinary field, it merges insights from materials science, microbiology, nanotechnology, and AMR, with a particular focus on microbial biofilm dynamics. We also discuss cutting-edge nano formulation strategies for conventional antimicrobial agents that enhance their therapeutic performance. This comprehensive overview is intended to support a broad audience of researchers working in biomaterials, biomedical nanotechnology, infection control, and the development of improved therapies for managing persistent and chronic wound infections. Prior literature has largely concentrated on a limited range of nanoparticle materials. This work provides a comprehensive analysis of developments in nanoparticle technologies encompassing a wider array of materials and offers a comparative perspective with conventional methodologies, an approach not addressed in earlier work. Moreover, this review uniquely adopts a One Health framework, emphasizing the interconnectedness of human, animal, and environmental health in the context of AMR. This multidisciplinary perspective is often lacking in existing reviews, which tend to focus on either clinical or material science aspects in isolation. By bridging gaps between public health, microbiology, and nanoscience, we aim to provide a holistic understanding of how nanotechnologies can be strategically leveraged across sectors to combat AMR.

1.1 Development of AMR

Some bacteria inherently possess resistance to antibiotics[26] due to altered membrane structure without any mutation. These modifications prevent antibiotics from interacting with or penetrating the membrane, rendering them ineffective. Gram-negative bacteria, in particular, exhibit higher resistance compared to Gram-positive bacteria due to their outer membrane[27,28] which restricts the

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penetration of large antibiotics through its small pores. Another form of intrinsic resistance occurs when bacteria lack specific membrane ligands, preventing antibiotics from attaching to and deactivating the bacteria[29]. However, bacteria can also acquire resistant traits through two other mechanisms: chromosomal mutations or gene transfer. Chromosomal mutation involves irreversible changes in the bacterial genome. When exposed to antibiotics, bacteria undergo evolutionary adaptation due to environmental pressures. Mutations that confer resistance traits are positively selected within bacterial populations and passed vertically to subsequent generations[30]. Over time, these mutations accumulate, providing robust resistance or even immunity against antibiotics. Certain species may develop resistance more rapidly based on their genomic mutation rates[31]. Moreover, if two antibiotics share similar structures or mechanisms of action, a mutation conferring resistance to one antibiotic can confer resistance to the other as well[13] leading to the simultaneous development of multiple antibiotic resistances.

AMR can also spread through HGT. When two different strains of bacteria coexist in the same microenvironment, genetic elements from one strain can be passed to the other independent of reproduction[18]. Transferable genetic elements include plasmids, phages and transposons[32]. Research has found that, unlike vertical inheritance, HGT does not require positively selecting environments and can occur in the absence of antibiotics[33]. HGT occurs through three pathways: transformation, transduction and conjugation. In 1928, it was demonstrated that certain bacteria possess the ability to uptake and express extracellular fragments of DNA[34]. This process, called transformation, allows the indirect exchange of genes between bacteria. The fragments involved are chromosomal and are not part of any transferable genetic elements. This ability has been observed in approximately 1% of bacteria species[35]. On the other hand, transduction and conjugation occur exclusively through transferable genetic elements[13]. Transduction occurs when an infecting bacteriophage accidentally uptakes bacterial plasmid or chromosomal DNA alongside the phage DNA. Upon infecting a new cell, the bacteriophage transfers the bacterial genes it has picked up to the host[36]. Several studies have shown that genes conferring resistance against antibiotics can be spread through transduction[37,38].

Conjugation facilitates the transfer of genes between bacteria through direct cell-to-cell contact *via* surface pili. Genes are transferred in the form of plasmids, which are collections of functional genes capable of self-replication organized into stable structures. Conjugative plasmids contain a distinct DNA segment known as the origin of transfer (oriT), which allows for the transfer of

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plasmids[39]. Once inside the recipient bacterium, these plasmids are expressed, allowing new traits to be inherited. Many genes responsible for antibiotic resistance have been identified on plasmids, facilitating their spread through conjugation[40].

1.2 Mechanism of AMR

1.2.1 Modification of Membrane Permeability

Antibiotics like β-lactams exert their effects by penetrating bacterial cells through the outer membrane. A common resistance mechanism involves reducing the permeability of this outer membrane. Antibiotics typically enter by utilising the porin family of proteins, which are embedded in the outer membrane. Numerous studies have shown that these proteins are often downregulated or modified with increased selectivity in resistant bacteria[41,42]. In contrast, bacteria like *Pseudomonas aeruginosa* form aggregates within self-produced matrices called biofilms. These biofilms consist of lipids, polysaccharides, and proteins, providing robust resistance against multiple antimicrobial agents[43].

1.2.2 Efflux Pumps

Efflux pumps are naturally occurring structures in bacteria that are responsible for the excretion of toxic compounds from within the cells. Through mutations, these pumps can adapt to specifically target antimicrobial molecules, enabling the rapid removal of these substances and thwarting their effects effectively[44]. Efflux pumps can target numerous antimicrobial agents, and mutated efflux pump genes can spread to other bacterial species *via* gene transfer mechanisms.

1.2.3 Enzymatic Inactivation

In addition to preventing antibiotic molecules from entering the cell, bacteria can develop resistance by directly modifying the antibiotics themselves, a process known as enzymatic inactivation. Several enzymes, including carbapenemases, β-lactamases and others, can hydrolyses various classes of antibiotic molecules, rendering them inactive[26]. A more common form of modification is the addition of functional groups to the antibiotics, which inhibit binding *via* steric hindrance. These enzymes, such as acyltransferases and phosphotransferases, form the largest family of resistance enzymes, collectively called group transferases[45].

1.2.4 Target Site Modification

Antibiotics exhibit high specificity for their target sites, and any changes to these targets can hinder effective binding (**Fig. 1**). Such modifications can take place in a multitude of ways. For example, rifampin is an antibiotic that binds to the β subunit of RNA polymerase enzymes. A single point

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mutation in the rpoB gene, which encodes RNA polymerase β, can alter the amino acid sequence, preventing rifampin from binding effectively and thus blocking its action[46]. Alterations can also occur through post-transcriptional modification of target proteins. Erythromycin ribosome methylase (*erm*) genes encode a family of enzymes that methylate a single nucleotide of the 23S rRNA in the 50S subunit of bacterial ribosomes. This modification confers resistance to macrolides and lincosamides antibiotics, which bind to the 23S rRNA[47].

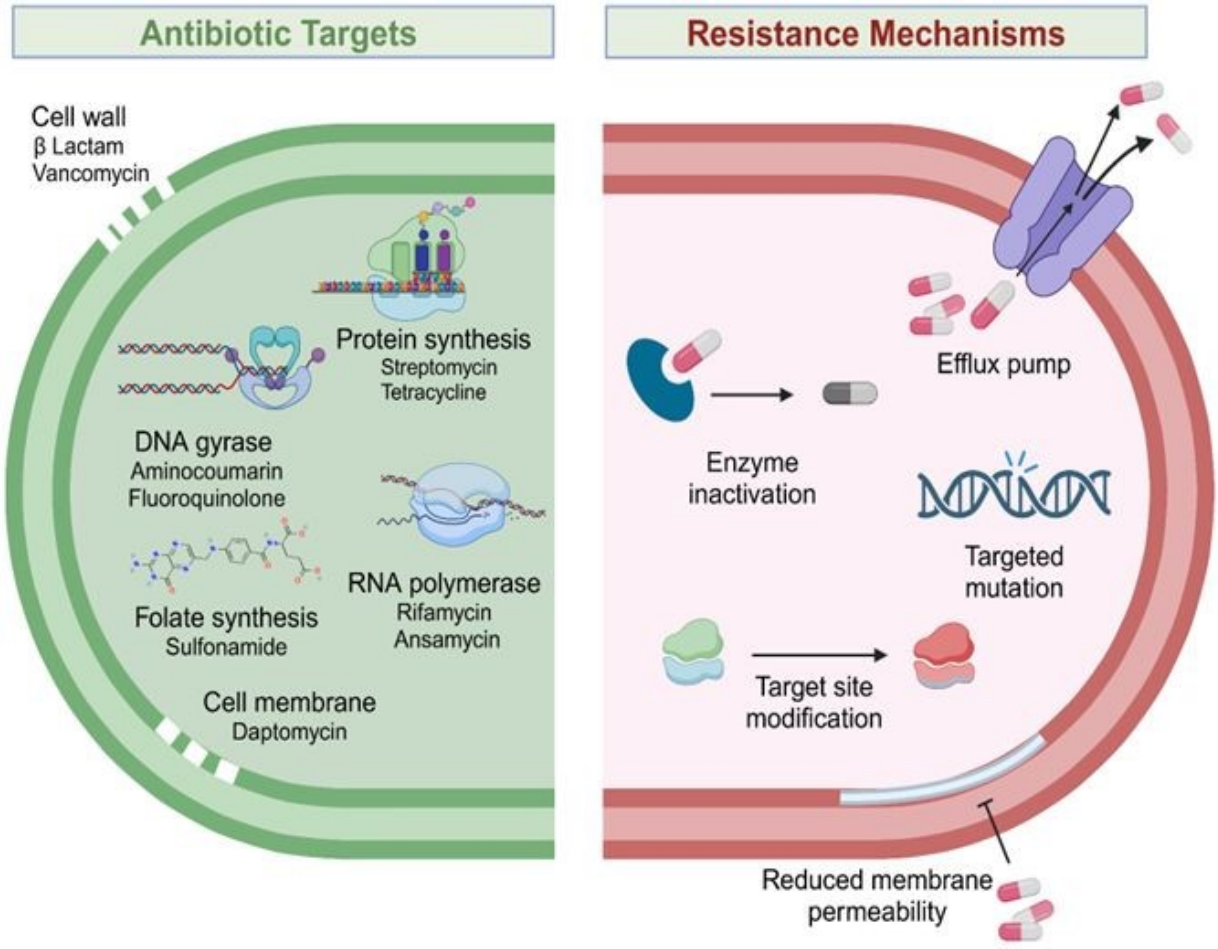

Figure 1: Schematic representation of common antibiotic targets and corresponding bacterial resistance mechanisms. The left panel illustrates key cellular targets of antibiotics within bacterial cells, including the cell wall (targeted by β-lactams and vancomycin), cell membrane (targeted by daptomycin), DNA gyrase (inhibited by aminocoumarins and fluoroquinolones), RNA polymerase (targeted by rifamycin and ansamycin), folate synthesis pathway (inhibited by sulfonamides), and protein synthesis machinery (targeted by agents like streptomycin and tetracycline). The right panel depicts major resistance strategies employed by bacteria to evade antibiotic effects, such as enzymatic inactivation of drugs, enhanced efflux pump expression to expel antibiotics, target site modification, reduced membrane permeability preventing drug entry, and accumulation of targeted genetic mutations. Together, these elements highlight the interplay between therapeutic

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intervention and microbial adaptation, underscoring the challenges in combating antimicrobial resistance.

2. AMR from a One Health Perspective

AMR represents one of the most pressing challenges in global health, and it is a prime example of why a One Health approach is urgently needed[48]. The One Health concept is a multidisciplinary and collaborative framework that recognizes the intricate link between human health, animal health, and the environment, aiming to develop integrated strategies to address complex health threats. The rise and spread of AMR are deeply rooted in interconnected sectors ranging from human healthcare and veterinary medicine to agriculture and environmental contamination[49,50]. Inappropriate and excessive use of antibiotics across these domains such as prophylactic use in livestock, overprescription in human medicine, and antibiotic pollution in soil and water systems has collectively accelerated the development of resistant microbial strains[51].

Historically, the roots of the One Health approach can be traced back to the 19th century, when Rudolf Virchow introduced the term zoonosis, highlighting the relationship between animal and human diseases. This foundation was later strengthened by Calvin Schwabe's concept of "One Medicine"**,** emphasizing the integration of veterinary and human health disciplines. The modern One Health agenda gained momentum following the 2004 Wildlife Conservation Society's Manhattan Principles, which called for cross-sectoral efforts to address emerging infectious diseases and ecosystem health[52].

In the context of AMR, the One Health approach brings much-needed clarity to how resistance genes and resistant organisms circulate among humans, animals, and the environment. For instance, antibiotic use in livestock for growth enhancement and disease control can lead to the emergence of resistant bacteria in animals, which may then be transferred to humans through direct contact or the food chain. Likewise, misuse in clinical settings such as incorrect prescriptions, incomplete treatment courses, and patient non-compliance can promote the survival and spread of resistant strains[53]. Moreover, environmental reservoirs, particularly water bodies contaminated by pharmaceutical effluents, agricultural runoffs, and untreated sewage serve as breeding grounds for resistance genes. These genes can be horizontally transferred among bacteria *via* mobile genetic elements, increasing the pool of resistant organisms across ecosystems. This microbial gene exchange can occur across species and habitats, creating a global AMR network that transcends geographical and biological boundaries.

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The cumulative effect of these interactions has made the prevention and treatment of bacterial infections increasingly difficult. Addressing this multifaceted threat requires coordinated action involving healthcare providers, veterinarians, environmental scientists, researchers, policymakers, and public health authorities. This review emphasizes that AMR cannot be tackled in isolation within any single sector. Instead, it must be approached through integrated, cross-disciplinary efforts that reflect the One Health ethos where collaboration between human, animal, and environmental health sectors becomes the foundation for sustainable and effective AMR mitigation strategies. **Fig. 2** highlights the complex exchange of resistant pathogens and resistance genes among humans, animals, farming systems, water sources, environmental reservoirs, and vectors. Key routes include hospital and community waste contributing to environmental contamination, sewage affecting water supplies, and the potential for transmission through food consumption, animal waste, irrigation, and direct contact with contaminated vectors. This multi-directional flow emphasizes the necessity for integrated surveillance and cross-sectoral collaboration to mitigate AMR emergence and spread[54].

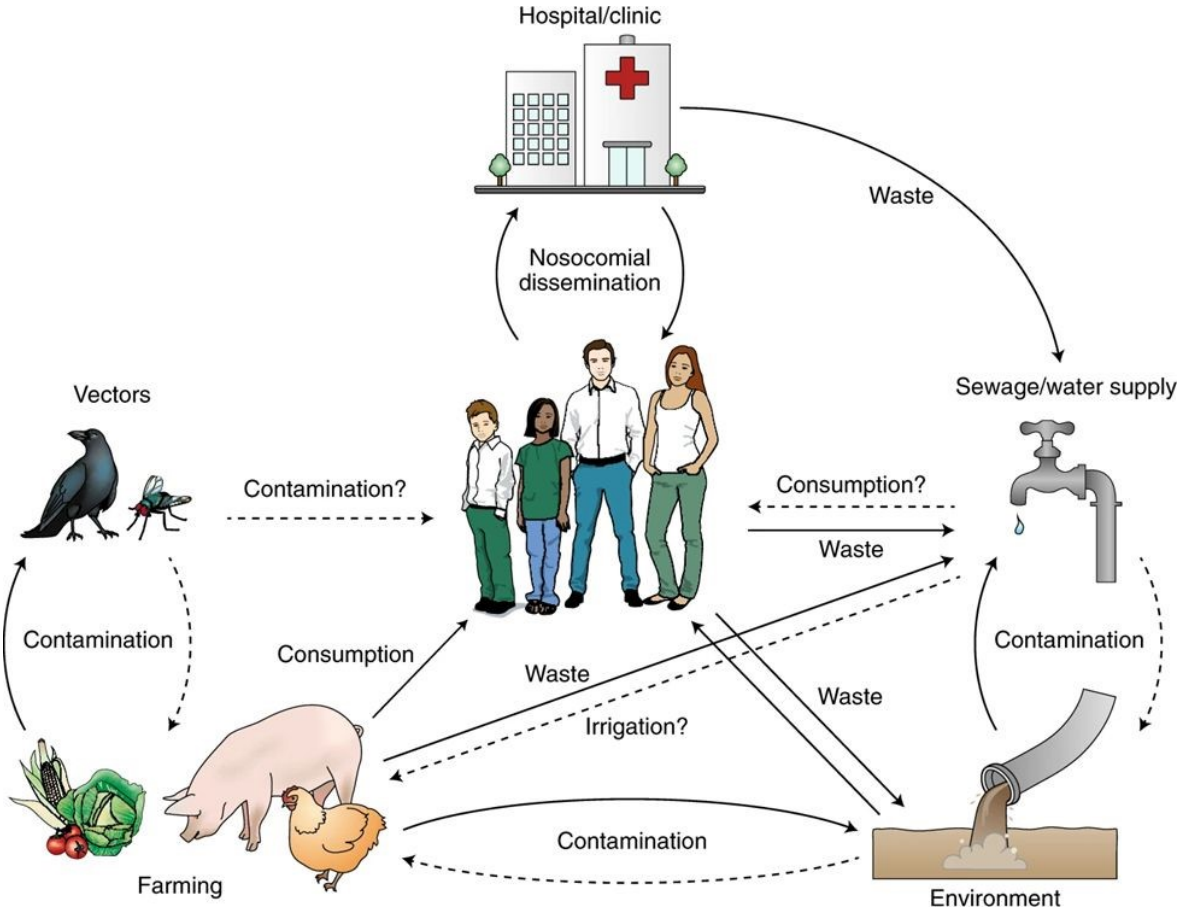

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Figure 2: Schematic illustration of the interconnected transmission pathways of AMR within the One Health framework (Reproduced with permission from Ref.[54], @ 2018, SpringerNature).

3. CURRENT APPROACHES TO COMBATING AMR

The increasing prevalence of AMR is a pressing concern for governmental and international bodies. Heightened awareness of this issue has spurred significant efforts to develop methods aimed at preserving the effectiveness of antimicrobials. This section will explore several conventional approaches currently under study as potential solutions, discussing their advantages and limitations.

3.1 Novel Drug Discovery

Following the discovery of penicillin, a wide array of antimicrobial agents were swiftly developed and integrated into medical practice, marking the 'golden age of antibiotics' that persisted until the 1960s. Progress slowed considerably thereafter, with few new antibiotic classes identified until the introduction of oxazolidinones and lipopeptides in the early 2000[55]. Presently, most newly developed antibiotics either belong to existing classes (e.g., dalbavancin, ceftaroline) or are combinations of established molecules[56].

Identifying novel antibiotic classes has become increasingly challenging, and the creation of entirely new antibiotic molecules has markedly declined. A report by the Infectious Diseases Society of America noted a 75% reduction in antibiotic approvals by the US Food and Drug Administration from 1983 to 2007[57]. This has been linked to several reasons, of which economic cost is a primary concern. The cost for research on new antibiotic classes is significant and has the potential for failure, due to which investing in such research carries a high risk for pharmaceutical industries. Consequently, many are focusing instead on strategies to enhance the efficacy of existing antibiotics, some of which show promising results.

3.2 Combination Drug Therapy

Combination Drug Therapy (CDT) involves simultaneously using multiple distinct antimicrobial drugs to treat a single infection. Research indicates that using antibiotic agents in combination enhances efficiency, reducing the likelihood of bacteria developing resistance mechanisms[58,59]. CDT has several other advantages over traditional single-antibiotic monotherapy. By combining drugs, a broader spectrum of species can be targeted, which is advantageous when the infectious agent is unidentified. Moreover, lower concentrations of each drug are needed, decreasing the risk of side effects and toxicity[60]. The selection of drugs plays a critical role in the effectiveness of

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CDT. If the antibiotics employed share identical or similar mechanisms, resistance developed against one could confer resistance to others. Hence, the antibiotics used in CDT must target distinct targets across different pathways, different aspects within a single pathway, or unique facets of a common target molecule[42,61]. However, this technique comes with a few drawbacks. Antibiotic molecules can interact unpredictably, causing unforeseen side effects and complications during treatment. Moreover, a meta-analysis of randomized controlled trials comparing β-lactam monotherapy and combination therapy found that while combination therapy had a lower treatment failure rate, it was also associated with higher mortality and fungal superinfection rates[62].

3.3 Bacteriophage-Based Therapy

Bacteriophages are viruses that specifically infect bacteria and are bactericidal in nature. These phages inject their genetic material into the host bacterium, where it replicates to form new phages. Upon maturation, these phages release lytic proteins that rupture the bacterial cell. Bacteriophages were first discovered in 1917[63] and were quickly recognized as potential therapeutic agents. Subsequent trials began to assess the feasibility of using phages in clinical settings. Unlike traditional antibiotics, which have a broad spectrum of activity, bacteriophages bind exclusively to specific receptors on bacterial cells, unique to each bacterial species. This highly host-specific action preserves beneficial bacteria in the gut and other microbiomes, which are often disrupted by antibiotic treatments[64]. Due to their self-replicating nature, even lower concentrations of phages are sufficient for the treatment. This allows the usage of smaller dosages, which reduces the risk of immunogenic responses[61]. Furthermore, since a single bacteriophage can lyse a single bacterial cell, there is no MIC for the treatment to be effective. Nevertheless, bacteria can still evolve resistance to bacteriophages, similar to antibiotics, by inhibiting different life stages of the virus[65]. The incorporation of bacteriophages into clinical treatment is hindered by their narrow host range. Identification of the infecting bacterial strain is necessary before phage therapy can be administered, unlike traditional antimicrobial agents that can be applied more broadly. Moreover, bacteriophages are specific to individual bacterial species, necessitating a mixture of phage species for the treatment of multiple bacterial infections. Currently, the use of bacteriophages in clinical settings is subject to numerous government regulations, due to which the scope for research on phage therapy is limited[66].

3.4 Antimicrobial Adjuvants

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Adjuvants are compounds that enhance the efficiency of antibiotics by increasing the susceptibility of target cells. Typically, devoid of antimicrobial activity themselves, adjuvants instead suppress bacterial resistance mechanisms[67]. Given the declining rate of new antimicrobial drug discoveries, there is growing interest in exploring adjuvants to augment the effectiveness of existing antibiotics. Adjuvants function by reversing or neutralizing the effects of resistance genes, thereby allowing antibiotics to act unhindered. Compounds like clavulanic acid and quercetin inhibit resistance enzymes such as β-lactamases and aminoglycoside phosphotransferases[68,69]. Certain D-amino acids can disrupt biofilm formation and dismantle existing biofilms[70]. Other observed methods of action include enhancing cellular uptake of antibiotics, inhibiting efflux pumps, and inducing oxidative stress in bacteria[61].

Despite promising outcomes, discovering new adjuvant molecules remains challenging. Identifying compounds that effectively target specific resistance mechanisms is both time-consuming and costly. Moreover, like combination drug therapy, interactions between adjuvants and antibiotics can lead to adverse side effects[71]. Therefore, intermolecular interactions must be thoroughly studied prior to clinical application. These challenges currently limit the widespread use of antimicrobial adjuvants.

3.5 Antimicrobial Peptides

Antimicrobial Peptides (AMPs) encompass a diverse group of natural peptide chains and proteins that exhibit antimicrobial properties. Found in virtually all organisms, AMPs are integral components of the innate immune system, with bactericidal action against foreign pathogens. In addition to their antimicrobial capabilities, these proteins can bolster host immune responses against infections[72]. The discovery of AMPs dates back to 1939 with the isolation of gramicidin from Bacillus bacteria, which was shown to effectively prevent pneumococcal infections in mice[73]. AMPs are typically short (<50 amino acids), highly hydrophobic, and carry net positive charges; hence, they are also referred to as Cationic Host Defense Peptides (CHDP)[74]. Most AMPs have a broad range of action, targeting most microorganisms, including bacteria, viruses and fungi.

Bacterial cell membranes, characterized by a negative charge from lipopolysaccharides and lipoteichoic acids, attract cationic antimicrobial peptides through electrostatic interactions. Once attached, the peptides penetrate the membrane due to their hydrophobic nature, increasing membrane permeability and leading to cell lysis[75]. AMPs may also disrupt the cytoplasmic membrane using similar mechanisms. Studies indicate that certain AMPs interfere with internal

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bacterial processes such as cell wall synthesis[76]. AMPs have also been shown to have antiviral properties, by inhibiting viral entry to cells or disruption of viral envelope and membranes[75]. Furthermore, AMPs can stimulate the host immune system through various mechanisms. For instance, AMPs like Cathelicidin act as chemoattractant, recruiting immune cells such as leukocytes and neutrophils[77]. Cathelicidin and hepcidin also promote the production of chemokines, which enhance the neutrophil response[78,79]. Wuerth et al. reported the ability of the peptide IDR-1002 to regulate *P. aeruginosa* lung infections and related inflammations[80]. Other AMPs have been found to influence cytokine-mediated pathways through inhibition of pro-inflammatory cytokines, enhance phagocytosis activity in macrophages, and indirectly facilitate recruitment of leukocytes[74].

Research indicates that the development of AMP resistance mechanisms is rare, as they act on multiple targets fundamental to the cell structure[75]. Experiments performed on multidrug-resistant bacteria by Veldhuizen et al. found that when AMP resistance does develop, it is temporary and not retained by subsequent generations in the absence of AMPs, suggesting that the development of major resistance is improbable[81]. Furthermore, while resistance between different AMPs has been reported, studies indicate that the emergence of cross-resistance between antibiotics and AMPs is unlikely to occur[82]. Despite these advantages observed *in vitro*, only a few antimicrobial peptides have gained clinical approval from the US Food and Drug Administration (FDA)[83]. This is mainly due to the proteins' low stability and their limited tolerance for salts and other human physiological compounds, which inhibit their efficacy *in vivo*[84]. Consequently, there is considerable interest in developing synthetic AMPs with enhanced stability. Studies have also highlighted the potential of AMPs to act as adjuvants alongside antibiotics[85]. **Fig. 3** provides a comprehensive chronological overview of the development of antibiotics, emergence of resistance, and the introduction of alternative therapies. The timeline begins with the early discovery of penicillin in 1928 and tracks the Golden Age of Antibiotics between the 1940s and 1960s, characterized by the introduction of broad-spectrum antibiotics such as streptomycin, tetracycline, and vancomycin. However, following this prolific period, there was a notable decline in the discovery of new antibiotic classes, coinciding with the increasing emergence of resistant bacterial strains such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE). **Fig. 3** also highlights the parallel evolution of alternative therapeutic strategies, including phage therapy, antimicrobial peptides, NPs, and monoclonal antibodies,

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which align with the growing urgency to combat AMR. The rise of extensively drug-resistant pathogens in the early 2000s further reinforces the pressing need for novel interventions. Recent advances, such as the development of NPs for antimicrobial drug delivery and successful personalized phage therapies, exemplify the integration of nanotechnology into the broader One Health strategy against AMR.

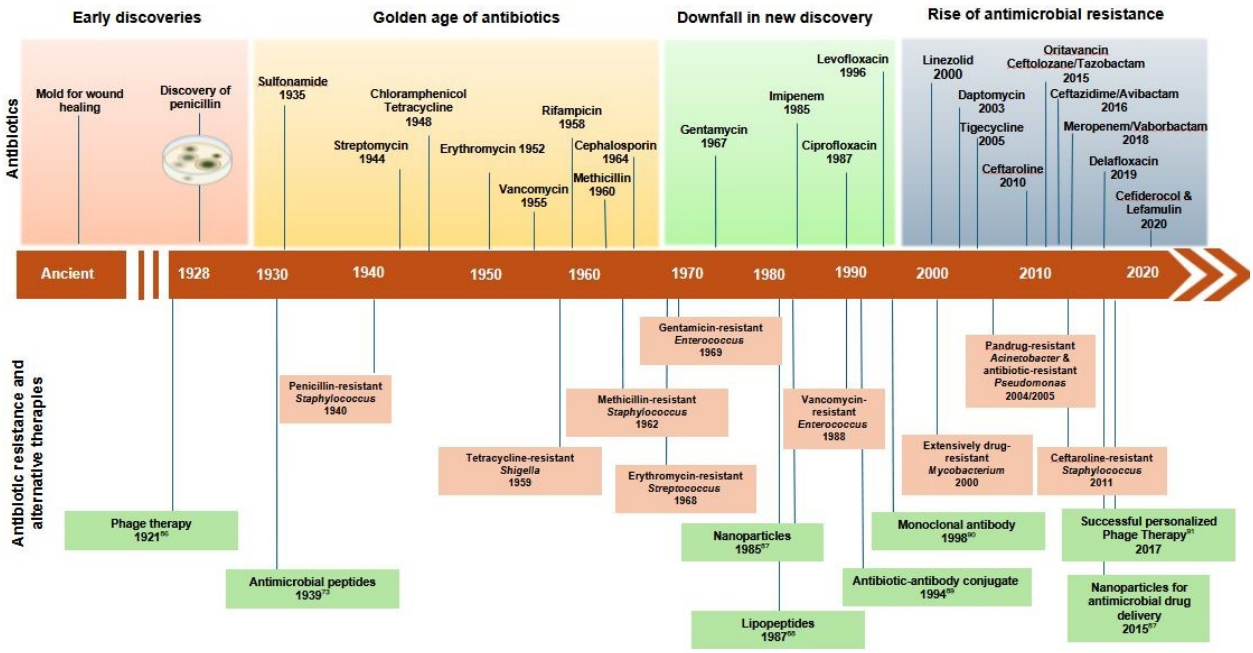

Figure 3: Timeline illustrating the evolution of antibiotic discovery, emergence of AMR, and the development of alternative therapeutic strategies. The upper panel highlights four distinct phases in antibiotic development: (i) early discoveries including the use of mold for wound healing and the discovery of penicillin in 1928, (ii) the "golden age of antibiotics" (1935-1960) characterized by the introduction of multiple broad-spectrum antibiotics such as sulfonamides, tetracyclines, and rifampicin, (iii) a decline in new antibiotic development from the 1970s to the 1990s despite the emergence of drugs like gentamycin and ciprofloxacin, and (iv) a modern phase (2000 onward) marked by the rise of antimicrobial resistance and the approval of last-resort or combination antibiotics like linezolid, tigecycline, and ceftazidime/avibactam. The lower panel tracks the parallel rise of resistance, such as penicillin-resistant Staphylococcus in 1940, methicillin-resistant strains in the 1960s, and vancomycin-resistant Enterococcus by 1988. It also presents alternative AMR combating strategies, including the advent of phage therapy (1921), antimicrobial peptides (1939), NPs (1985), monoclonal antibodies (1998), and antibiotic-antibody conjugates (1994). Recent advancements include successful personalized phage therapy (2017) and nanoparticle-based drug delivery systems (2015), underlining innovative responses to the escalating AMR crisis. This figure underscores the urgent need for integrated strategies in the face of stagnating antibiotic discovery and growing resistance. Referenced from [73,86–92]

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4. NANOTECHNOLOGY-BASED APPROACHES TO AMR

Nanotechnology involves the study and synthesis of materials whose dimensions range between 1 nm and 100 nm, known as NPs. Within this size range, materials exhibit unique biological and chemical properties that can be harnessed for various applications[93]. NPs offer several advantageous properties that position them as promising alternatives to conventional antibiotics. Their physicochemical characteristics, such as size, shape, surface charge, and composition, significantly influence their interactions with both microbial pathogens and host tissues[94–96]. Notably, NPs with smaller dimensions exhibit a higher surface area-to-volume ratio, enhancing their ability to interact with bacterial membranes and penetrate host tissues, thereby improving antimicrobial efficacy and targeted delivery. The shape of NPs also plays a critical role; for instance, rod-shaped particles often demonstrate superior cellular penetration compared to spherical ones. Surface charge further dictates biological interactions- positively charged NPs are more likely to bind to negatively charged bacterial membranes, leading to membrane disruption and cell death. Additionally, surface charge can affect cellular uptake by host cells. The material composition of NPs, whether metallic or organic, along with surface modifications such as conjugation with antibodies, peptides, or other functional groups, can modulate their antimicrobial performance, enhance biocompatibility, and reduce cytotoxicity. Nanotechnology is increasingly being applied in medicine to address the growing challenge of drug resistance, particularly through the targeted delivery of therapeutic agents. Notably, NPs composed of metals such as gold, silver, and zinc oxide (ZnO) have demonstrated significant efficacy against antibiotic-resistant bacteria, including strains like MRSA, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli. The use of NPs for antimicrobial delivery offers several key benefits: enhanced bioavailability at the site of infection, reduced systemic toxicity, and the ability to maintain therapeutic concentrations even at lower doses, thereby minimizing side effects and slowing resistance development. Certain NPs, such as silver, selenium, and engineered carbon dots, demonstrate antimicrobial properties[97]. This antimicrobial activity includes a wide range of targets, including inhibition of biofilm formation[98], release of reactive oxygen species (ROS), inhibition of mRNA transcription and disruption of cell membranes (**Fig. 4**)[99]. Moreover, NPs serve as effective carriers for antibiotics due to several advantageous characteristics. They are resistant to AMR mechanisms, thus shielding the drug from inhibitory processes. Their small size facilitates easy uptake by bacteria through phagocytosis, ensuring rapid action. Furthermore, targeting NPs to

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specific receptors allows for localized antibiotic delivery, minimizing potential side effects[100]. Studies have shown enhanced antibiotic efficacy when combined with various types of NPs[101]. Ongoing research explores NPs as a promising strategy to combat AMR. This section will discuss notable types of NPs currently under investigation for this purpose. **Table 1** contains detailed information about the antimicrobial activity of some well-known nanomaterials.

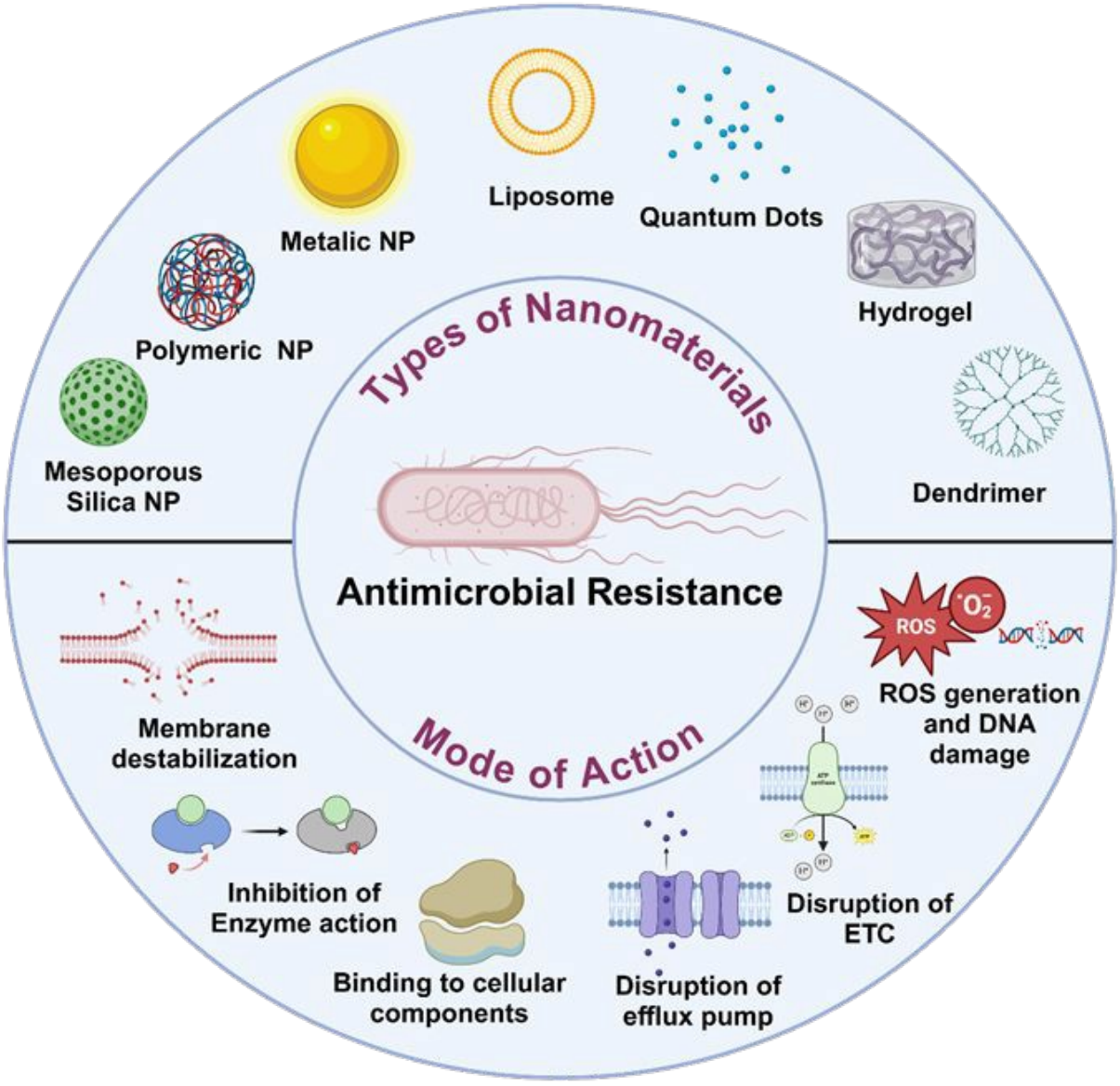

Figure 4: Schematic representation highlighting the various types of nanomaterials and their antimicrobial modes of action against resistant pathogens. The upper semicircle illustrates diverse nanomaterial platforms including metallic NPs, liposomes, quantum dots, hydrogels, dendrimers, mesoporous silica NPs, polymeric NPs, and others, each possessing unique physicochemical properties that contribute to antimicrobial activity. The lower semicircle summarizes the primary mechanisms by which these nanomaterials exert antimicrobial effects. These include: (i) destabilization of microbial membranes, (ii) inhibition of essential enzymes, (iii)

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interaction with and binding to cellular components, (iv) interference with bacterial efflux pump systems, (v) disruption of the electron transport chain (ETC), and (vi) generation of ROS, leading to oxidative stress and DNA damage. Collectively, these multifaceted mechanisms enable nanomaterials to combat drug-resistant microorganisms effectively, offering promising alternatives to conventional antibiotics in the fight against AMR.

4.1 Chitosan

Chitosan (CS) is a natural biopolymer composed of a linear polysaccharide chain, which can disrupt bacterial cell membranes. It is obtained by the alkaline or enzymatic deacetylation of chitin; a biopolymer found in *Crustacean* exoskeletons. The deacetylation process creates C_2 amino acids, which are positively charged in acidic conditions (below pH 6.5)[102]. These positively charged groups interact with the negatively charged lipopolysaccharides and lipoteichoic acids present in bacterial membranes, increasing their permeability and causing leakage of cellular contents[103]. The increased permeability allows CS to penetrate the cell membrane, where it binds to microbial DNA and inhibits protein synthesis by blocking mRNA transcription[104]. Experiments conducted by Y. Ma et al. suggest that CS also inhibits the action of metalloproteins by chelation of metal ion cofactors, which are necessary for microbial growth and proliferation[105]. At lower concentrations, CS demonstrates bacteriostatic activity rather than bactericidal effects. As the negative charge of the cell membrane is highly conserved in both Gram-positive and Gram-negative bacteria, the development of resistance against CS is considered improbable[99]. However, this antibiotic activity of CS is reliant on numerous factors, including solubility and pH, and is unreliable in variable conditions[105].

Numerous studies have demonstrated the antiviral and antifungal properties of CS as well[102]. In the NPs scale, CS has a high surface charge-to-volume ratio. This increases its affinity for bacterial membranes, resulting in higher penetration. Yadav et al. reported a sensitive, non-invasive, and label-free strategy for detecting trace amounts of norfloxacin (NF) in human urine using a nanocomposite composed of nanostructured yttrium oxide (nY_2O_3) and CS. The composite material was synthesized *via* a low-temperature, single-step hydrothermal method, offering a straightforward and cost-effective approach for fabricating the sensor platform[106]. CS NPs were shown to have more antimicrobial activity than normal CS and certain antibiotics, including doxycycline[107]. CS's polymeric nature allows it to be shaped into nanostructures as desired to improve the delivery mechanism. In addition, augmenting polymeric NPs with antibiotic molecules or other NPs allows them to be released in a controlled, targeted manner[108]. These

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composite structures also show a greater inhibitory effect on microbes than their components. Ibrahim et al. demonstrated that CS NPs loaded with gentamycin sulphate, ciprofloxacin HCl, and tetrachlorocycline increased the zone of inhibition of the antibiotics against *E. coli* and *S. aureus* by 16.5 mm, 15.5 mm and 7-10 mm, respectively[109]. In addition, Sobhani et al. found that ciprofloxacin-loaded NPs have a 50% lower MIC than regular ciprofloxacin (CPX)-HCl molecules[110]. Another study showed that the addition of CS to silver molecules increased the diameter of the inhibition zone against bacteria by approximately 15 mm, saturating at 70% CS content[105]. Fayed et al. conducted an experiment demonstrating that amoxicillin, when encapsulated within CS NPs, exhibited a biphasic release profile, with approximately 33% of the drug released within the first 2 hours and an additional 33% released over the subsequent 70 hours[111] **[Fig. 5(2)]**. Recently, CS has also been reported to enhance the antimicrobial activity of cloxacillin[112], dihydroartemisinin[113] **[Fig. 5(1)]**, and ampicillin[114] against methicillin-resistant *S. aureus* strains. CS also acts as a carrier and stabilizer for copper-containing NPs and nitric oxide-releasing NPs[115]. Among all polymeric NPs being studied, CS has demonstrated the greatest potential for use against AMR. CS NPs were found to increase efficacy of β-lactam antibiotics and β-lactamase inhibitors against β-lactam resistant pathogens[116]. Polymeric CS is already used in a variety of medical, agricultural and pharmaceutical fields; however, CS NPs are yet to be approved for clinical use[117].

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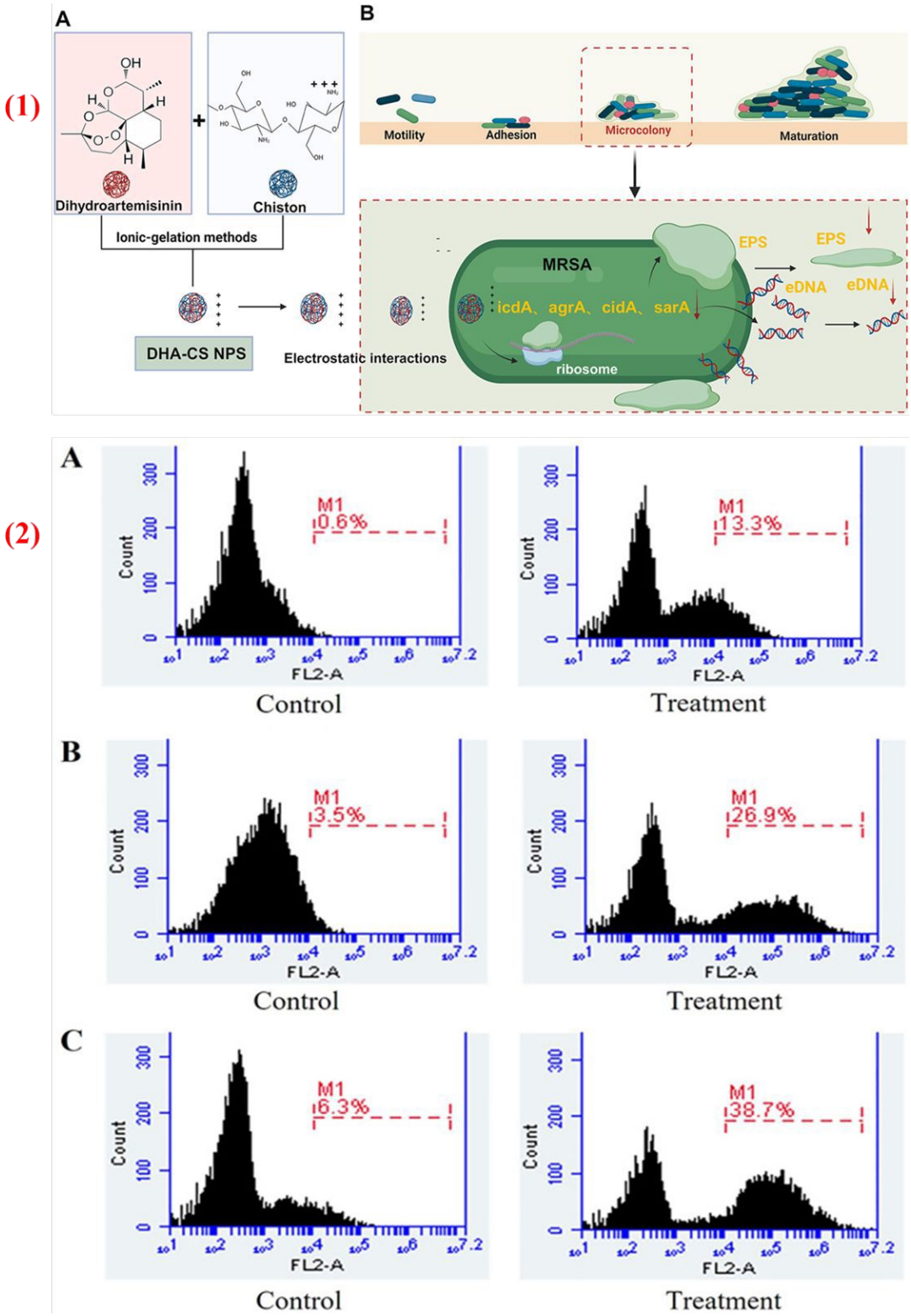

Figure 5: (1) Schematic illustration of the synthesis and antibacterial mechanism of DHA-CS NPs against MRSA biofilms. (A) Dihydroartemisinin (DHA) and CS are combined using ionic-gelation methods to synthesize DHA-loaded CS NPs (DHA-CS NPs). (B) Biofilm formation stages of MRSA include motility, adhesion, microcolony formation, and maturation. DHA-CS

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NPs interact with MRSA, penetrating the cells and downregulating key biofilm-associated genes (icdA, agrA, cidA, sarA) (Reproduced with permission from Ref.[113], @ 2025, Elsevier]; **(2) Flow cytometry analysis of time-dependent internalization of Cur-CS NPs by *Helicobacter pylori*.** Representative flow cytometry plots illustrate the nanoparticle uptake by H. pylori after incubation for 4 hours (A), 8 hours (B), and 24 hours (C). Untreated H. pylori cells under identical conditions served as negative control. Signal detection was performed using the FL2 channel (green fluorescence), with linear amplification (FL2-A) and doublet discrimination applied to ensure the analysis of single-cell events (Reproduced with permission from Ref.[111], @ 2023, Elsevier].

4.2 PLGA NPs

PLGA (Poly[lactic-co-glycolic] acid) is a synthetic polymer frequently used for synthesizing NPs. When hydrolyzed, PLGA degrades into lactic acid and glycolic acid, which can be metabolized by human bodies. Hence, it is considered to be biodegradable and biocompatible as well[118]. Studies have shown that PLGA NPs are not cytotoxic to macrophages and other immune cells[119,120] **[Fig. 6(1)]**. Due to these properties, PLGA is frequently used for drug delivery in clinical trials, particularly for targeting intracellular infections. Alsa'd et al. successfully utilized PLGA NPs to deliver ciprofloxacin and ceftazidime to macrophages infected with *Staphylococcus aureus* and *Klebsiella pneumoniae*[121]. Sabaeifard et al. demonstrated that Amikacin-loaded PLGA NPs provide sustained release for up to 9 hours after incubation *in vitro*, effectively delivering the drug in a controlled manner without a significant reduction in activity[119]. PLGA NPs have also been successful in targeting and delivering Gentamicin, which typically has poor cell penetration, to organs affected by brucellosis[122] and in treating *K. pneumoniae* infections[123] using PLGA NPs **[Fig. 6(2)]**. Other antibiotics demonstrating enhanced antimicrobial activity when delivered using PLGA include CPX[124] **[Fig. 7(1)]**, rifampicin[125] **[Fig. 7(2)]**, azithromycin[126,127] **[Fig. 6(4)]**, nafcillin and sparfloxacin[118]. Furthermore, PLGA NPs can be functionalized to improve the specificity of delivery and disassemble biofilm obstructions. Baelo et al. showed that ciprofloxacin-loaded PLGA NPs functionalized with the enzyme DNase I were able to eradicate up to 95% of established *P. aeruginosa* biofilms, and repeated administration eradicated up to 98%[128] **[Fig. 6(3)]**. PLGA NPs have demonstrated stability under varying pH and temperature conditions; however, long-term stability studies are still ongoing[129]. Numerous studies have found that antibiotics delivered using PLGA NPs are protected from bacterial resistance mechanisms[120,130,131]. The US Food and Drug Administration and the European Medicine Agency have approved the use of PLGA NPs for parenteral administration of antibiotics[130].

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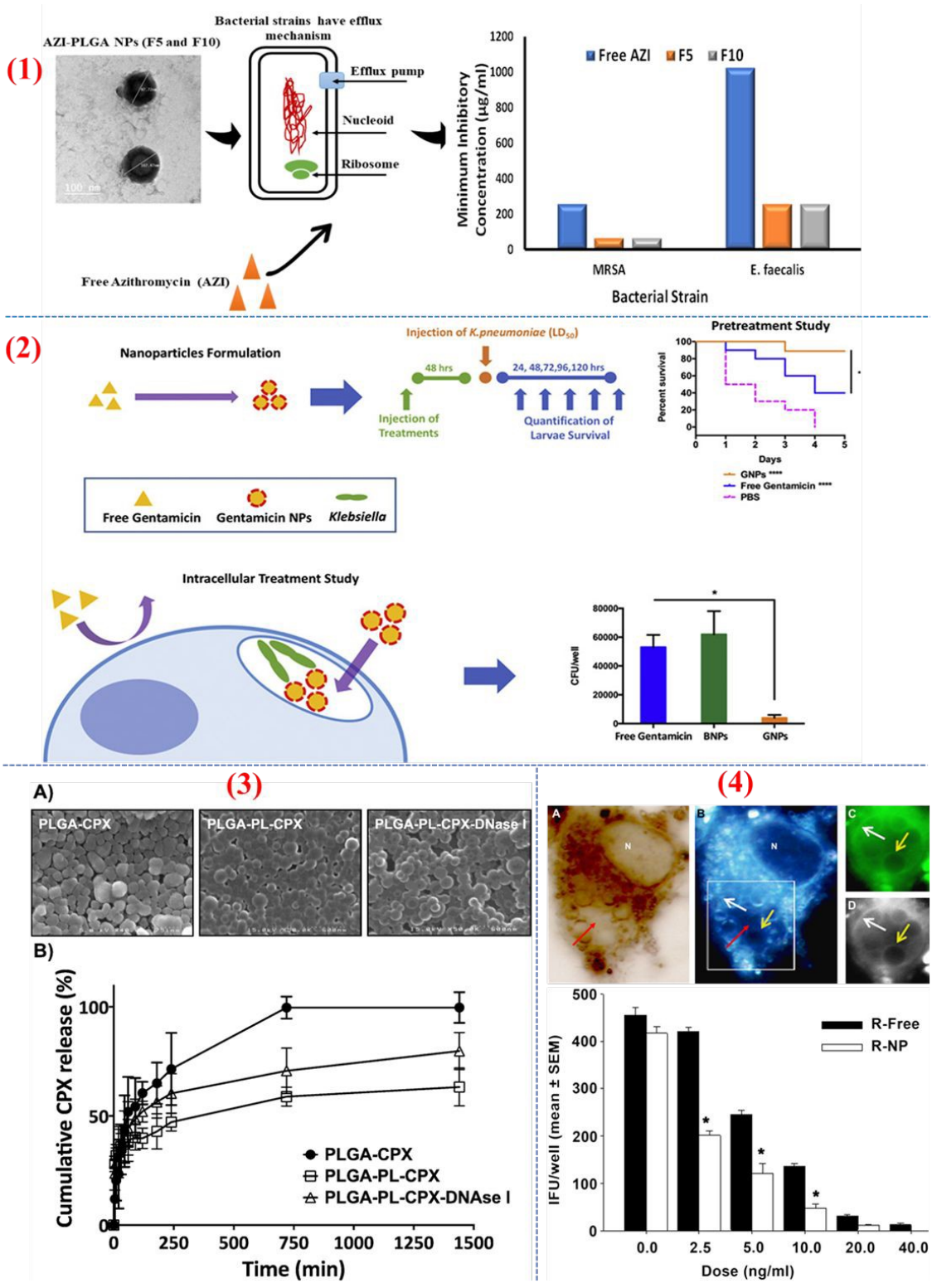

Figure 6: (1) Characterization and antibacterial efficacy of AZI-loaded PLGA NPs against resistant bacterial strains (Reproduced with permission from Ref.[120], @ 2022, MDPI); **(2)** Schematic representation of evaluation of gentamicin-loaded NPs for enhanced antibacterial efficacy against Klebsiella pneumoniae (Reproduced with permission form Ref.[123], @ 2018,

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Elsevier); **(3)** Physicochemical characterization and drug release profile of nanoparticle formulations. (A) SEM images depicting the surface morphology of different nanoparticle systems: PLGA–CPX, PLGA coated with polydopamine and loaded with ciprofloxacin (PLGA–PL–CPX), and PLGA–PL–CPX further functionalized with DNase I. (B) Comparative release profiles of CPX from the respective nanoparticle formulations, illustrating variations in drug release kinetics based on surface modification and enzyme conjugation (Reproduced with permission from Ref.[128], @ 2015, Elsevier); and **(4)** Representative images demonstrate the selective localization of labeled NPs inside cellular inclusions associated with persistent infection, indicating effective cellular uptake and homing capability of the nanoparticle system toward infected compartments (Reproduced with permission from Ref.[126], @ 2011, Elsevier).

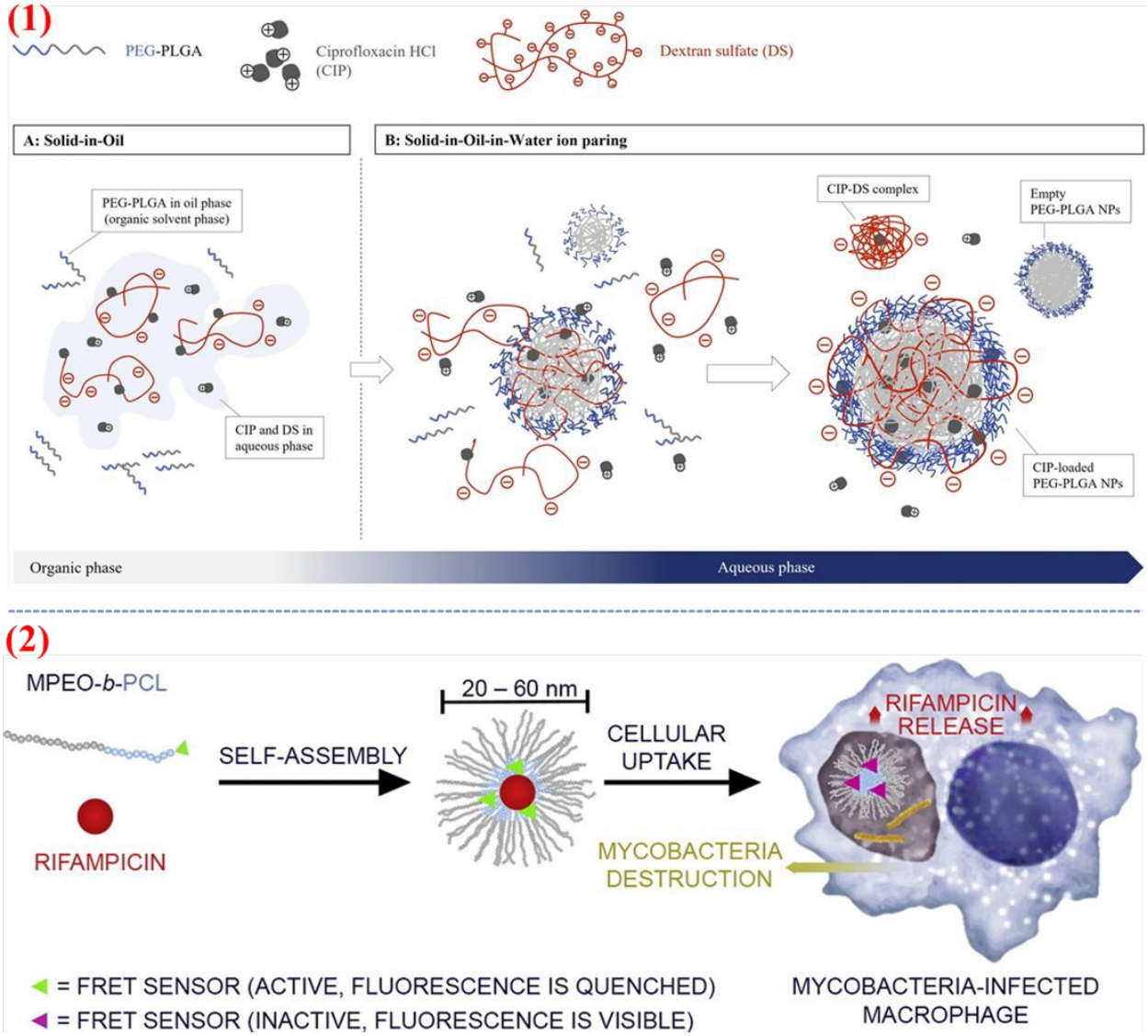

Figure 7: (1) Schematic representation of the synthesis of ciprofloxacin-loaded PEG–PLGA NPs using the solid-in-oil-in-water (S/O/W) ion-pairing technique. (A) In the initial solid-in-oil (S/O) step, ciprofloxacin (CIP) is dispersed in a PEG-PLGA polymer solution prepared in acetone, followed by the addition of dextran sulfate to facilitate ion pairing. (B) The resulting

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mixture is then introduced into distilled water, leading to spontaneous nanoparticle formation through a self-assembly process (Reproduced with permission from Ref.[124], @ 2023, SpringerNature); and **(2)** Schematic of rifampicin-loaded polymeric NPs for targeted delivery to mycobacteria-infected macrophages (Reproduced with permission from Ref.[125], @ 2017, Elsevier).

4.3 Liposomes

Liposomes are vesicles consisting of one or more types of lipid bilayers enclosing a spherical aqueous cavity. They are one of the most extensively studied NPs for drug delivery. By adjusting the concentration of different lipids in the liposome, properties such as surface charge, size, membrane fluidity and stability can be tailored as desired, offering significant flexibility in optimizing the delivery mechanism[132]. The outer surface can be positively, negatively or neutrally charged. Positively charged liposomes show the highest affinity for the negatively charged bacterial membranes. Additionally, liposomes can also be modified by incorporating proteins, oligosaccharide chains, antibodies or immunoglobulins to target particular ligands[133,134]. This modification enhances specificity, ensuring that non-target microbes remain unaffected. Furthermore, the composition of the liposomal formulation can significantly influence its antimicrobial efficacy. Natsaridis et al. reported that increasing the cholesterol content within the liposomal membrane enhanced the activity of moxifloxacin against *Staphylococcus epidermidis* biofilms[135].

Liposomes can effectively deliver both hydrophobic and hydrophilic molecules. They are not targeted by microbial resistance mechanisms and hence protect antimicrobial molecules until delivery[136]. Research has demonstrated that liposomes enhance the persistence of antibiotics, significantly extending their stability and controlling the rate of diffusion from the vesicle. For instance, ciprofloxacin-loaded liposomes were found to be active up to 48 hours after initial injection[137]. Additionally, liposomes exhibit low toxicity and reduce the cytotoxic effects of antibiotic agents[136]. Studies indicate that liposomes enhance the efficacy of antimicrobial agents against resistant bacterial strains, particularly those that modify their cell surfaces, such as *P. aeruginosa*. For example, lipomycin encapsulated tobramycin has shown increased bactericidal effect even at sub-MIC concentrations[138], and was able to disrupt existing biofilm structures of *P. aruginosa*[139]. Other antibiotics, including ofloxacin[140], polymyxin B[141], and aminoglycosides[142], have also retained antibacterial activity at sub-MIC concentrations when delivered *via* liposomes. In the context of antimicrobial-resistant pathogens, liposomal delivery of rifabutin has been shown

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to significantly reduce the MIC against methicillin-resistant *S. aureus*[143]. However, the use of liposomes for drug delivery has some limitations, including low drug loading capacity, potential antibiotic leakage and physicochemical instability[136]. In addition, the development and production of new liposomes can be prohibitively expensive. Despite these constraints, numerous liposome-based products are available for clinical use.

4.4 Solid Lipid NPs

Solid Lipid NPs (SLNPs) were developed as an alternative to liposomes, aiming to enhance stability, minimize drug leakage, and improve biodegradability[144]. The biocompatibility of these NPs is significantly greater than that of many other nanocarriers currently under investigation[145]. In addition, SLNPs face fewer regulatory constraints and can be scaled up easily. These NPs consist of colloidal forms of high-melting-stable lipids produced through emulsion or ultrasound dispersion in water or a solvent with similar properties[136]. Due to their small size, they can bypass cell membranes and enter the cell *via* endocytosis, allowing direct delivery of the antimicrobial molecule[132]. SLNs have a higher entrapment efficiency than liposomes and are better suited for long-term storage. Studies have shown that the entrapment efficiency of drugs like tetracaine and etomidate in SLNs ranges from 85 and 99%[146]. Furthermore, ciprofloxacin-incorporating SLNs remained stable for up to 9 months at both 4°C and 25°C[147]. Research indicates that drug molecules can undergo either a burst release (as seen with tetracaine and etomidate[144]) or a sustained release (as with CPX[148]), depending on the composition of the SLNPs and the nature of the antibiotic. Taheri et al. demonstrated that although free vancomycin, ampicillin, and CPX exhibited greater inhibitory effects during the initial 24 hours, their solid lipid nanoparticle (SLNPs)-encapsulated counterparts achieved superior overall inhibition over a 72 hour period[149]. Notably, SLNs improve the activity of antibiotics such as rifampin against biofilm-producing resistant bacterial strains, potentially by inhibiting efflux pumps and protecting drugs from modifying enzymes[150]. Despite their advantages over liposomes, SLNPs still face limitations, particularly concerning low loading capacity. To address this issue, Nanostructured Lipid Carriers (NLCs) are being explored as a potential solution[151]. NLCs are composed of an unstructured matrix polymer that contains either amorphous or imperfect cavities, which allows for improved loading capacity and reduced risk of drug leakage. Additionally, they can be rigidified during storage without compromising entrapment efficiency[152]. However, some studies have reported cytotoxic effects associated with (NLCs), indicating the need for further investigation into their biocompatibility[145]. In a pioneering

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effort, Yadav et al. engineered a label-free electrochemical immunosensor utilizing molybdenum disulfide NPs ($nMoS_2$ NPs) immobilized on an indium tin oxide (ITO) coated glass surface for the selective detection of ampicillin[153]. Building on this work, the same group later designed a sensitive immunosensing platform for gentamicin detection by integrating multiwalled carbon nanotubes (MWCNTs) with MoS_2 into a nanocomposite matrix. This nanocomposite was electrophoretically deposited onto an ITO substrate, enhancing both the conductivity and surface area of the sensor for improved analytical performance[154].

4.5 Metal and Metal Ion NPs

Metal ion NPs form one of the largest categories of NPs currently under investigation, as they possess considerable antibacterial activity, typically bactericidal in nature. Due to their positive charge, they can interact with and disrupt the outer membrane and cell wall of the bacteria. In addition, metal ion NPs have been found to promote metal ion release, reactive oxygen species generation[136], and denaturation of nucleic acids[155] in bacterial cells. The size of these NPs allows them to penetrate the cells *via* endocytosis and membrane pores[136]. Multiple studies have reported the development of resistance against metal NPs[156,157]. However, these were found to be the result of multiple cooperating mutations, making their simultaneous occurrence unlikely. Furthermore, metal ion NPs serve as effective carriers for antibiotics. When coupled with these NPs, antibiotics demonstrate enhanced antimicrobial activity, reduced MIC, and improved specificity[101].

4.5.1 ZnO NPs

ZnO NPs are of significant interest in medical and pharmaceutical fields due to their anti-inflammatory and antimicrobial properties, and they have also been researched for potential use in cancer therapy as well[13]. These NPs exhibit low MIC against *S. aureus* and *S. typhimurium,* but they are notably less effective against *P. aeruginosa*[158]. A 2022 study demonstrated that ZnO NPs exhibited potent activity against several β-lactam-resistant bacterial strains, including *Escherichia coli* and *Salmonella typhi*, primarily through ion-induced oxidation of metabolic components and the β-lactamase enzyme. Notably, a bactericidal effect was observed only at concentrations exceeding 0.24 mg/mL[159]. Research indicates that ZnO may be less effective against Gram-negative bacteria, possibly due to the presence of thick outer memberanes[160]. Additionally, ZnO NPs have been shown to disrupt the biofilm formation in regular strains and methicillin-resistant strains of *S. aureus*[161,162].

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A 2010 study by Thati et al. investigated the interaction of ZnO NPs with 25 different antibiotics, measuring their efficacy against *S. aureus*. Most antibiotics tested, including β-lactams, cephalosporins, aminoglycosides, and tetracyclines, showed increased inhibition zones during disk diffusion, with penicillin, amoxiclav, and amikacin demonstrating the most significant improvements. In contrast, norfloxacin and clarithromycin exhibited minimal increases in efficacy[163]. However, the same year, Banoee et al. reported that amoxicillin and penicillin G displayed decreased activity against *S. aureus*, while ciprofloxacin showed a 27% increase in its inhibition zone[164]. Experiments by Ghasemi & Jalal found that ZnO NPs significantly increased the zone of inhibition of ceftazidime against *A. baumannii* to 33 nm, contrasted to 0 nm for the free antibiotic molecule[165]. Shokrollahi et al. similarly demonstrated improved efficacy for cefotaxime, with the highest inhibition occurring at a concentration of 32 μg/mL[166]. This could be attributed to ZnO NPs disrupting the function of NorA proteins, which are essential components of efflux pumps in *S. aureus*[167]. Meropenem delivered *via* ZnO NPs was shown to completely inhibit biofilm formation by *P. aeruginosa*, accompanied by a significant downregulation of biofilm-associated gene expression. *In vivo* administration of this formulation effectively cured *P. aeruginosa*-induced keratitis in rat models[168]. ZnO NPs have been classified as "Generally Regarded as Safe" by the US Food and Drug Administration for use in food and agricultural industries, owing to their low toxicity and high biocompatibility.

4.5.2 Silver (Ag) NPs

Historically, Ag has been used as an antimicrobial agent since at least 1000 BC. A study in 1869 revealed that Ag could exert antimicrobial effects at very low concentrations[169]. Since then, the use of Ag ions and Ag salts has become widespread in pharmaceutical and agricultural fields. Ag NPs also demonstrate strong bactericidal properties and are increasingly used in medicine, electronics and sanitation as antimicrobial agents[170]. Although the precise mechanism behind the antibacterial activity of Ag NPs is not yet fully understood, several theories have been proposed. Similar to other metal ions, Ag NPs can interact with and penetrate bacterial cell membranes, altering their structure and increasing permeability. Analysis of Ag NPs action on *E. coli* revealed the formation of 'pits' in the cell wall, in which NPs accumulated[171]. These enhance the uptake of Ag ions into the cell, where generation of reactive oxygen species[97], denaturation of ribosomes, and alteration of DNA have been observed[172,173]. Ag NPs exhibit strong inhibitory effects against yeast (MIC 6.6 nM - 3.2 nM) and *E. coli* (MIC 3.3 nM - 6.6 nM), while showing only mild

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inhibition towards *S. aureus* (MIC 33 nM). The rate of growth inhibition is dependent on the concentration of Ag[97]. Furthermore, Ag NPs have demonstrated antimicrobial activity against resistant bacteria strains, including methicillin-resistant *S. aureus* and methicillin-resistant *Staphylococcus epidermidis*[174]. Studies combining Ag NPs with antimicrobial peptides have demonstrated a synergistic effect, resulting in enhanced antimicrobial activity of both components against methicillin-resistant *S. aureus*[175]. These NPs can also serve as carriers for antibiotic drugs. For instance, when amoxicillin was delivered using nano Ag, its MIC decreased from 0.525 mg/ml to 0.150 mg/ml[176]. Ag NPs loaded with ciprofloxacin showed reduced MIC and a decrease in resistance-generating mutations in *P. aeruginosa*, with effects enhanced by adjuvants such as CS and betamethasone[177]. Ciprofloxacin-loaded Ag NPs also exhibited enhanced antibacterial efficacy against *Acinetobacter baumannii* and *Serratia marcescens*, producing inhibition zones of 36 mm and 40 mm, respectively[178]. Additionally, vancomycin, erythromycin, and penicillin G also showed increased antibacterial activity against *S. aureus*[179]. Cytotoxicity assessments conducted on a range of in vitro and in vivo models, including human macrophages, HeLa cells, and mouse stem cells, revealed no cytotoxic effects at bactericidal concentrations of Ag NPs. However, higher concentrations were found to reduce cell viability[180]. Further, Zheng and colleagues developed a nanocomposite consisting of lanthanum hydroxide integrated with graphene oxide (La@GO), which demonstrated a potent synergistic antibacterial effect against multiple resistant bacterial strains[168]. Notably, prolonged exposure of antimicrobial-resistant E. coli to sub-inhibitory concentrations of La@GO did not induce any measurable development of secondary resistance. In contrast, repeated treatment with conventional antibiotics or Ag NPs resulted in a significant increase in bacterial tolerance, ranging from 16 to 64 fold [**Fig. 8**].

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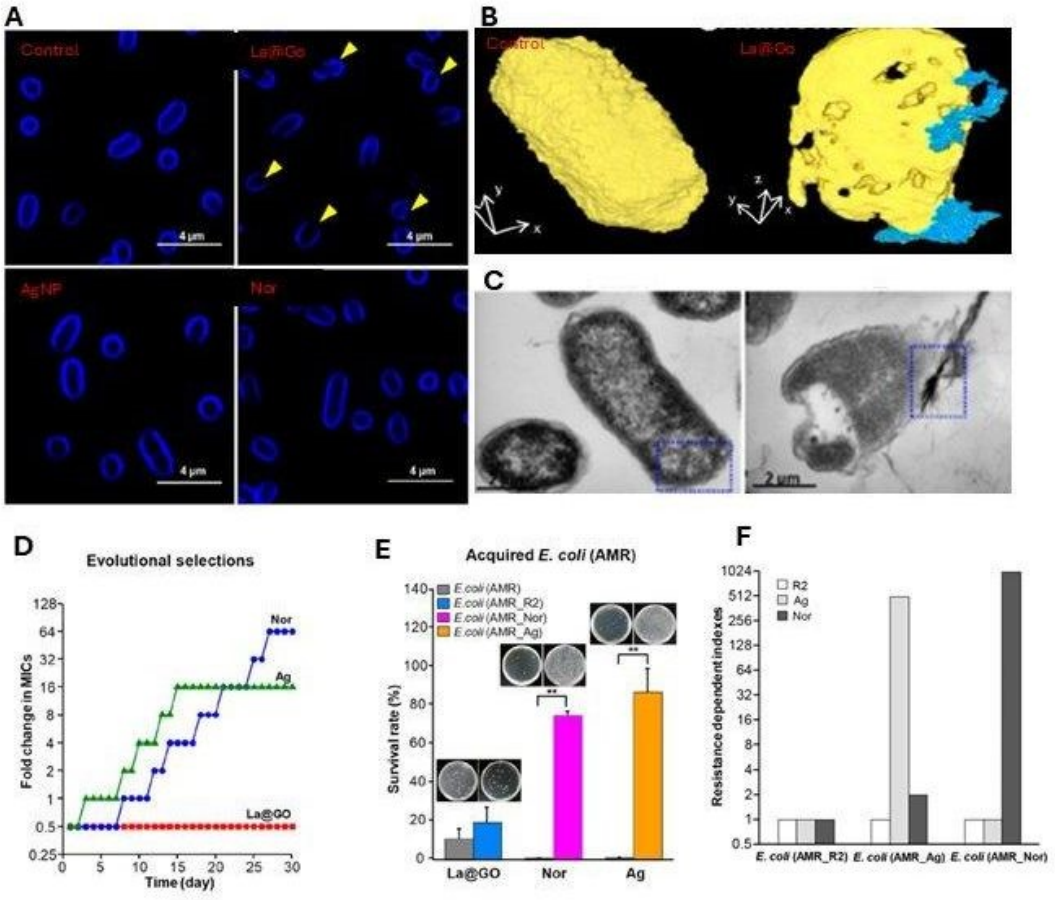

Figure 8: Engineered Lanthanum hydroxide and graphene oxide NCs (La@GO/R2) inhibit the Evolution of AMR. (**A)** Confocal microscopy images showing the peptidoglycan layer of multidrug-resistant (MDR) E. coli labelled in situ with a fluorescent D-amino acid after treatment with La@GO, Ag NPs, and norfloxacin; (**B)** Nano-CT scans of *E. coli* treated with La@GO; (**C)** Transmission electron microscopy (TEM) images of antimicrobial-resistant E. coli treated with La@GO; (**D)** The trajectory of AMR evolution in MDR E. coli was tracked by exposing the bacteria to La@GO, Ag NPs, and Nor for 30 days at MIC; (**E)** Development of resistance exposure to La@GO, AgNPs and Nor; and (**F)** Evaluation of cross-resistance development in E. coli after 30 days of exposure (Reproduced with permission from Ref.[181], @ 2019, American Chemical Society).

4.5.3 Copper (Cu) NPs

Due to the wide availability of Cu, Cu NPs are being researched as another alternative for AMR. However, their usage is limited as they oxidize rapidly in the presence of air or aqueous media, which significantly decreases their stability and antimicrobial ability[182]. Similar to other metal NPs, Cu NPs also form 'pits' in the bacterial cell wall, allowing them to accumulate and penetrate the cell, while destabilizing the stability of the bacterial membranes[183]. A 2013 study revealed the

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presence of reactive oxygen species and degraded DNA molecules in *E. coli* cells treated with Cu NPs. It was suggested that oxidation of cupric ions promotes the generation of the reactive oxygen species, which subsequently causes DNA degradation and protein oxidation[184]. Cu NPs have demonstrated efficacy against antimicrobial-resistant pathogens, with Wang et al. reporting activity against methicillin- and vancomycin-resistant *Staphylococcus aureus* strains[185] **[Fig. 9(2)]**. They also contribute to biofilm disruption, inhibiting 49% and 59% biofilm formation in *Klebsiella oxytoca* and *E. coli* bacterial strains, respectively[186].

Cu NPs have a synergistic effect with antibiotics, and the efficiency of both is found to increase in combination. A study exploring the action of different antibiotics in the presence of Cu NPs found that the antibacterial activity of ampicillin, amoxicillin and ciprofloxacin was increased against multiple Gram-positive and Gram-negative species, including *E. coli*, *M. luteus*, and *S. typhi*. In the same study, gentamycin showed only a mild increase in activity[187]. Cu NPs also benefit when co-delivered with other metallic NPs. A 2023 study demonstrated that the combined administration of Cu and Ag NPs significantly improves the performance of both[188] **[Fig. 9(1)]**. A growing concern about the use of Cu NPs is their cytotoxicity. NPs can easily penetrate organs through their membranes due to their size. While data is limited, a study reported that accumulation of Cu NPs in mice caused damage to multiple organs, including the liver, kidney and spleen[189]. Similarly, another study focusing on nasal administration of NPs to mice found that repeated exposure caused inflammation of lung tissues and degradation of nasal epithelial cells[190].

4.5.4 Gold (Au) NPs

The antimicrobial effects of Au NPs are multi-faceted and target numerous aspects of the cell. By interacting with the cell wall and cell membrane, these NPs increase the membrane permeability and cause leakage of cellular contents. Proteins such as adenosine triphosphate synthetases and nicotinamide adenine dinucleotide dehydrogenases are inhibited, leading to a disruption of metabolic activities and death of the bacterial cell. Furthermore, they prevent the binding of ribosomes to tRNA, blocking the membrane synthesis mechanism. Au NPs also enhanced chemotaxis and attacked phosphorus-containing sites in DNA molecules[191]. A 2016 study examined the effects of Au NPs on the growth of various bacteria species, reporting a growth reduction of 53.19% in *S. aureus*, 68.1% in *E. coli*, 49.4% in *B. subtilis*, and 13.8% in *K. pneuomoniae*[192]. When conjugated with Au NPs, the antibiotics streptomycin and kanamycin demonstrated a significant decrease in MIC against *E. coli*, *M. luteus*, and *S. aureus*, while

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amoxicillin showed only a slight decrease[193]. Additionally, ampicillin-coated Au NPs exhibited enhanced antimicrobial activity against methicillin-resistant *S. aureus* and drug-resistant strains of *P. aeruginosa* and *E. aerogenes*[194]. Delafloxacin, a fluoroquinolone antibiotic, exhibited enhanced antibacterial activity when delivered *via* Au NPs, with MIC reduced by 12.77 μg/mL against *Escherichia coli*, 13.51 μg/mL against *Pseudomonas aeruginosa*, 22.52 μg/mL against *Staphylococcus aureus*, and 33.21 μg/mL against *Bacillus subtilis*[195]. Vancomycin, too, experienced up to a 1.8-fold increase in efficacy against *E. coli*, *K. oxytoca*, and *P. aeruginosa*[196]. Khan et al. reported that β-caryophyllene-functionalized Au NPs effectively disrupted a mixed biofilm formed by fungal *C. albicans* and bacterial *S. aureus* species, in a concentration dependent manner[197] **[Fig. 9(3)]**. These NPs can also be functionalized with polymers, such as CS. Oligosaccharide-capped Au NPs were demonstrated to inhibit biofilm development and disrupt existing biofilm structures in *P. aeruginosa*[198]. One key advantage of Au over other metal ion NPs is the absence of reactive oxygen species generation, which reduces the toxicity towards mammalian cells while retaining bactericidal abilities[191]. The chemical inertness of Au further enhances its biocompatibility, allowing for easy functionalization with different ligands to improve specificity[13]. However, there is insufficient data regarding interactions and kinetics of Au NPs *in vivo*, largely due to a lack of clinical trials[199].

4.5.5 Titanium NPs

Titanium NPs possess great potential as antimicrobial agents due to their high stability and low risk of corrosion[136]. These NPs act by progressively increasing the permeability of the cell membrane, causing a significant leakage of intracellular components such as potassium ions, RNA and proteins. Microscopic analysis showed additional degradation of the cell wall and outer membrane. Reactive oxygen species are also produced through oxidative stress under stimulation by UV light. These properties target DNA molecules and proteins involved in metabolic processes, causing bactericidal effects[200]. Titanium oxide NPs were found to inhibit biofilm synthesis in methicillin-resistant *Staphylococcus aureus* and disrupt existing biofilm formations[161]. The conjugation of titanium NPs with ZnO NPs enhances the antimicrobial activity of both against gram-negative *E. coli* and *P. aeruginosa*, as well as gram-positive *S. aureus* and *B. subtilis*[201]. A study investigating the effect of titanium NPs on 23 antibiotic molecules found that the zone of inhibition increased for all antibiotics tested, including penicillin, amikacin, ampicillin, and gentamycin, except for nalidixic acid, which showed minimal improvement[202].

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Research suggests that titanium may have potential cytotoxicity at the nanoparticle scale. A 2010 report from the International Agency for Research on Cancer classified titanium dioxide (TiO_2) as a potential carcinogen, noting sufficient evidence of carcinogenicity in animals but insufficient data in humans[203]. Further analysis of titanium's cytotoxicity is necessary before it can be considered for clinical trials.

4.5.6 Magnetic NPs

Magnetic NPs are a distinct group characterized by similar physicochemical properties, primarily composed of iron oxides, iron-metal oxides or their derivatives. These NPs are particularly intriguing due to their superparamagnetic properties, which enable controlled delivery through magnetic fields[204]. Studies involving *E. coli* in the presence of iron NPs have reported increased oxidative stress and the generation of reactive oxygen species *via* the Fenton reaction. This causes the disruption of metabolic processes and inhibition of protein synthesis, leading to cell death[205]. Iron NPs exhibit bactericidal effect against multiple bacterial species, including *S. aureus*, *E. coli*, *P. aeruginosa, S. typhi* and *P. multocida*; often with MICs lower than most antibiotics. Notably, they show greater efficacy against Gram-positive as compared to Gram-negative bacteria[206] **[Fig. 9(4)]**.

Research on the delivery of streptomycin using CS-coated magnetic NPs demonstrated enhanced activity against Gram-negative *E. coli* and *P. aeruginosa*[204]. Functionalized iron oxide NPs were found to be effective at disrupting biofilm formation of *E. coli and S. aureus*[207]. Additionally, NPs composed of cobalt ferrite ($CoFe_2O_4$) and barium ferrite ($BaFe_{12}O_{19}$) exhibited antimicrobial activity against both *E. coli* and *S. aureus,* as well as fungal pathogens like *C. albicans* and *F. oxysporum*[208]. Vancomycin-conjugated magnetic NPs significantly reduced the MIC from 250–4000 μg/mL for standard vancomycin molecules to 13-28 μg/mL for the conjugated form, with membrane permeabilization observed within 2 hours, which was not detected for non-conjugated vancomycin[209]. Research on mouse fibroblast cells indicates that at low concentrations, magnetic NPs do not show cytotoxicity[210]. The US FDA has approved the use of a few magnetic NPs for applications in MRI imaging and cancer therapy[211]; however, further research is needed to explore their potential as antimicrobial agents.

4.5.7 Cobalt NPs

The use of cobalt NPs for antimicrobial treatment has gained significant interest over the past decade following the discovery of their antimicrobial properties[212]. Cobalt, similar to most metallic

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ions, is bactericidal, and exerts its biological effects primarily through the disruption of cellular membranes, generation of reactive oxygen species, and interference with protein synthesis and transcription[213]. However, the available data is still limited and insufficient for advancing clinical trials. Dogra et al. showed that cobalt-containing oxide and hydroxide NPs dramatically reduced the number of colony-forming units (CFUs) of *S. aureus* by 72%-98% in overnight culture[214]. When tested against various Gram-positive and Gram-negative bacteria, cobalt oxide NPs exhibited low MIC of 31.25 µg/ml against *E. coli* and *S. aureus*, while the MIC against *P. aeruginosa* was higher at 250 µg/ml. In contrast, the antibiotic gentamicin outperformed all samples, with a MIC of 10 µg/ml. Additionally, UV illumination significantly enhanced the antibacterial activity of cobalt NPs across all tested instances[215]. Interestingly, another study indicated that cobalt NPs surpassed gentamicin, cefotaxime, ciprofloxacin, and amoxicillin in effectiveness against multidrug-resistant *E. coli*, and were only outperformed by ciprofloxacin in multidrug-resistant *S. aureus*[216]. Moreover, *in vitro* tests of cobalt NPs demonstrate low cytotoxicity, with peripheral blood mononuclear cells showing over 80% cell viability up to 300 µg/mL, and are considered safe for non-cancerous human cells[217]. Nevertheless, further research on biocompatibility and *in vivo* kinetics is essential before initiating clinical trials for cobalt nanoparticle-based drugs.

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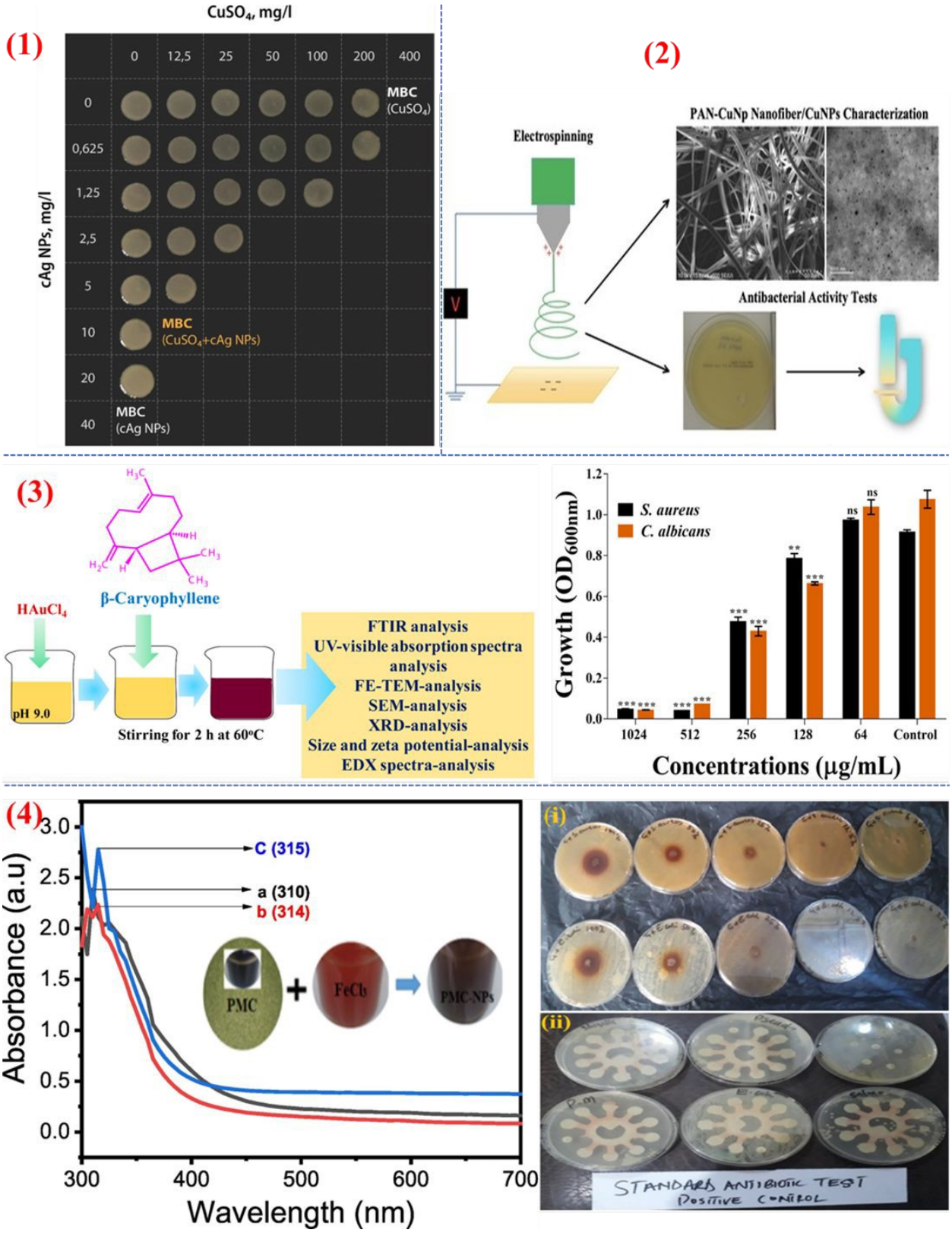

Figure 9: (1) Illustration of synergistic antibacterial activity of Cu sulfate ($CuSO_4$) and colloidal Ag NPs (cAg) against *Escherichia coli* K12 (Reproduced with permission from Ref. [188], @ 2023,

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SpringerNature); **(2)** Schematic representation of the fabrication and evaluation of PAN-Cu nanoparticle-based nanofibers and their antibacterial performance through *in vitro* microbiological assays to determine their potential against bacterial pathogens (Reproduced with permission from Ref.[185], @ 2022, MDPI); **(3)** Schematic overview of the synthesis and characterization of β-cyclodextrin-stabilized Au NPs (β-c-AuNPs) and their antimicrobial evaluation against Staphylococcus aureus and Candida albicans, demonstrating their broad-spectrum antimicrobial potential (Reproduced with permission from Ref.[197], @ 2023, MDPI); and **(4)** UV-Vis absorption spectra of NPs synthesized using P (a), M (b), and PMC (c) indicate successful formation and distinct optical properties of each formulation. The antimicrobial efficacy of PMC-NPs was further validated through agar diffusion assays, showing clear inhibition zones against Escherichia coli and Staphylococcus aureus (i), with comparison to a standard positive control (ii) (Reproduced with permission from Ref.[206], @ 2019, Elsevier).

4.5.8 Other Metallic NPs

Numerous other metal ion NPs have been studied for potential use as antimicrobial agents. However, research on these NPs is limited. Selenium NPs, for instance, inhibited the growth of *S. aureus* (63% inhibition) and *E. coli* (46% inhibition) after 24 hours of incubation, with maximum inhibition at 50 ppm concentration. The study also performed cytotoxicity assays on the NPs, reporting that after increasing the concentration to 50 ppm, 70% of human cells remained viable[218]. Lara et al. demonstrated the ability of CS-coated selenium NPs to disrupt pre-formed biofilms in the fungus *C. albicans,* reducing the IC_{50} from 21.7 ppm (naked NPs) to 3.5 ppm[219]. Ridha et al. showed that selenium NPs conjugated with levofloxacin and amikacin exhibited MICs 10–20 times lower than those of the free antibiotics against *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *E. coli*, indicating enhanced antibacterial efficacy and efficient drug delivery[220]. Geoffrion et al. found that Methicillin-resistant *S. aureus* and multidrug-resistant *E. coli* underwent dose-dependent inhibition in response to naked selenium NPs, with MIC values of 14.26 and 2.35 ppm, respectively. This indicates potential bacteriostatic and bactericidal effects that can be applied against drug-resistant bacteria. However, *in vitro* cytotoxicity assays were only conducted up to 1 ppm concentration, and the toxicity of MIC concentrations was not determined[221]. Huang and colleagues developed a rapid, one-step synthesis method to produce Se NPs functionalized with the antimicrobial peptide ε-poly-L-lysine (ε-PL). These engineered NPs exhibited a strong positive surface charge, contributing to their potent antimicrobial activity. The ε-PL-Se NPs were highly effective against a broad spectrum of microbial pathogens, including both Gram-positive and Gram-negative bacteria such as *E. coli, S. aureus, E. faecalis*, and *P. aeruginosa* as well as their

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drug-resistant strains and the fungal species *C. albicans*. The minimum fungicidal concentration (MFC) of 10PL-Se NPs over *C. albicans* was 26 ± 10 μg/mL[222] **[Fig. 10]**.

Cadmium Oxide NPs have been shown to have a significant inhibitory effect on *S. aureus*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* bacteria grown in nutrient agar media. This effect was reported to be greater than that of Cu NPs[223]. A similar study with cadmium sulfide NPs demonstrated concentration-dependent inhibition of *E. coli* and *S. aureus* colonies, however the zone of inhibition was smaller than that of regular tetracycline molecules[224]. While cadmium ferrite NPs were reported to have no notable activity on Gram-negative bacteria, calcium cadmium ferrite NPs inhibited the growth of both Gram-positive and Gram-negative bacterial species[225].

Silica NPs do not possess antibacterial properties; rather, they serve as carriers for antibiotics or work synergistically with other metals. For example, penicillin loaded into Ag-silica NPs reduced the MIC against methicillin-resistant *S. aureus* from 335 μg/ml to 130 μg/m[226]. Additionally, the delivery of vancomycin and polymixin B *via* mesoporous silica NPs enhanced the antibacterial activity and lowered the MIC against five species of bacteria: *E. coli*, *S. aureus*, *K. oxytoca*, *A. baumannii*, and *P. aeruginosa*[227]. Ciprofloxacin-loaded silica NPs inhibited biofilm formation of *Salmonella typhimurium* by 50% and reduced the MIC to 0.03125 mg/L[228].

Chaudhary et al. developed a highly sensitive and selective electrochemical immunosensor utilizing lanthanum oxide NPs (nLa_2O_3 NPs) for the detection of trace levels of the antibiotic ciprofloxacin (CPX)[229]. In a subsequent study, the group introduced an advanced biosensing platform incorporating a nanocomposite of nLa_2O_3 NPs anchored onto reduced graphene oxide (nLa_2O_3 NPs@rGO). This label-free sensor exhibited enhanced performance for the efficient electrochemical detection of CPX, benefiting from the synergistic properties of the nanomaterials used[230]. Cadmium, silica, selenium and lanthanum NPs are the most prominent among the numerous metal ions under investigation for antimicrobial activity. However, data regarding *in vivo* activity, cytotoxicity, long-term effects, and potential side effects is limited. Until further studies are conducted, clinical trials for the treatment of infections cannot be performed[136].

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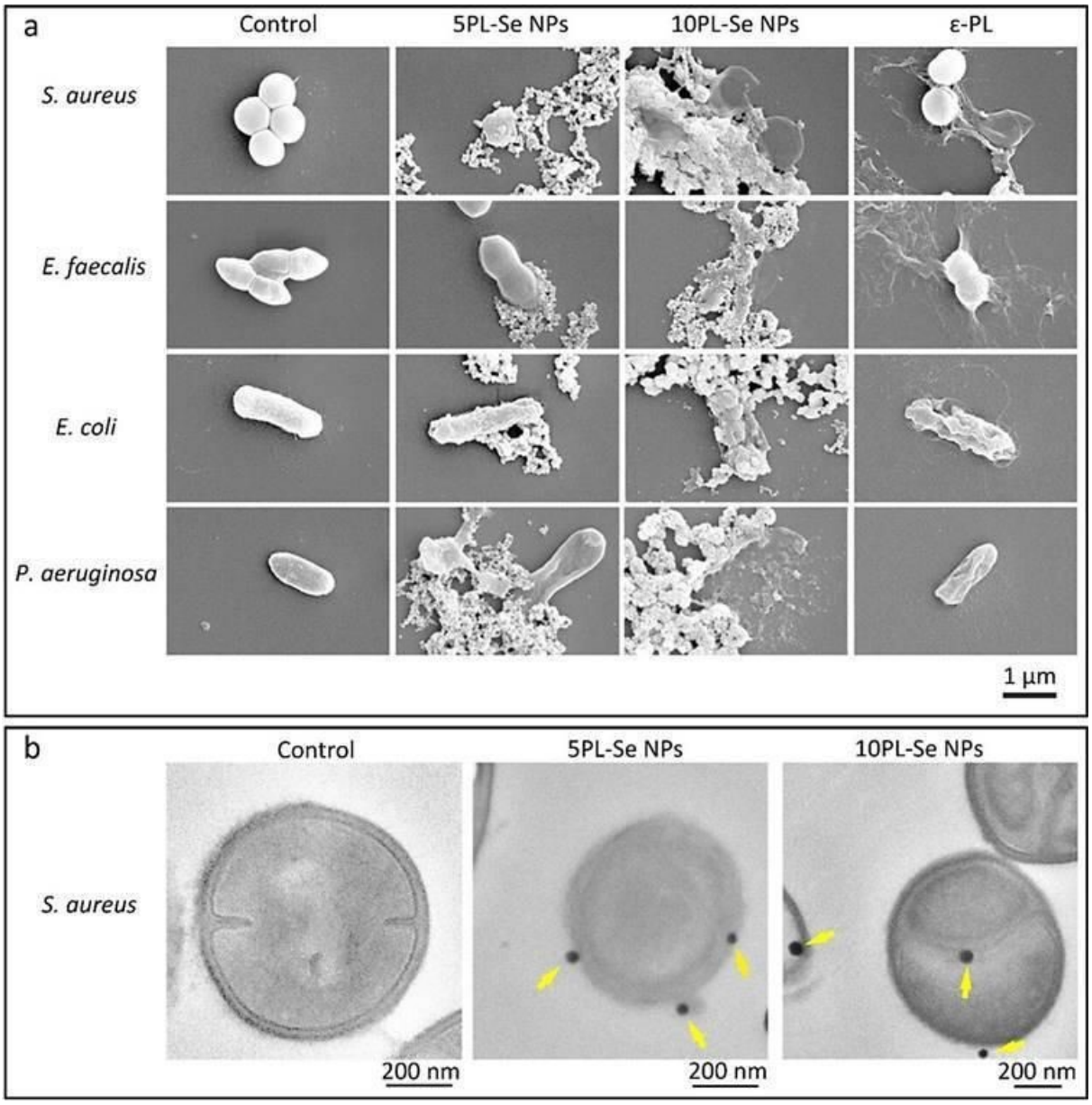

Figure 10: In a study, polylysine-coated NPs showed bactericidal activity against numerous gram-negative and gram-positive bacteria the including *E. coli, S. aureus, E. faecalis*, and *P. aeruginosa*. Here, 5PL-Se NPs, 10PL-Se NPs denote 5mg/ml, 10 mg/ml polylysine-coated selenium NPs, whereas ε-PL denotes antimicrobial peptide-poly-L-lysine stabilized Se NPs (Reproduced with permission from Ref.[222], @ 2024, American Chemical Society).

4.6 Carbon Dots

Carbon dots (CDs) are carbon NPs that are characterized by their fluorescent properties and surface functionalization. They are small (less than 10 nm in diameter) and are used in a variety of fields, including cell imaging and drug delivery[231]. They were first discovered in 2004[232] and quickly generated interest due to the presence of numerous surface groups, which allowed conjugation

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with a wide range of ligands and molecules. These NPs also possessed high stability and low toxicity, making them perfect for use in medical fields[233]. A simple and eco-friendly one-step hydrothermal approach was employed to synthesize blue-fluorescent carbon dots using the pulp extract of Ziziphus mauritiana as a natural carbon source. These biogenic CDs were utilized as an optical probe for the sensitive detection of the antibiotic ciprofloxacin[234]. It has been demonstrated that carbon dots reduce inflammation by preventing the generation of pro-inflammatory cytokines. One study reported that the anti-inflammatory effect of aspirin-conjugated carbon dots was higher than that of aspirin molecules alone[235]. The antibacterial effects of carbon dots were first reported in 2016[236], and are primarily bactericidal. They interact with the cellular barrier of bacteria and fungi through electrostatic interactions, causing damage to the cellular membranes. In addition, they promote the generation of reactive oxygen species, which further destabilizes the cell wall and causes cellular leakage. Carbon dots were also found to inhibit protein synthesis mechanisms, disrupt electron transport in the membrane[237], and bind with DNA molecules and destroy their secondary structures[238].

The antimicrobial activity of carbon dots was displayed against *E. coli* and *S. aureus*, with greater inhibition observed for *E. coli*[239]. Carbon dots are loaded with levofloxacin, an antibiotic to which pathogens typically develop rapid resistance. demonstrated enhanced activity against multiple species od *Staphylococcus* bacteria, including methicillin-resistant *S. aureus*[240]. The conjugation of ciprofloxacin with carbon dots resulted in a significant enhancement of the antibiotic's effectiveness, achieving a loading capacity of 90%[241]. Additionally, amine-terminating carbon dots functionalized with ampicillin increased antibacterial activity by generating additional reactive oxygen species. The presence of the target *E. coli* cells was detectable through UV-stimulated fluorescence of the carbon dots[242]. Carbon dots have also shown promise against antimicrobial-resistant infections. A 2020 study revealed their ability to inhibit multiple drug-resistant *Enterococcus* bacteria[243]. Furthermore, another study demonstrated that penicillin-conjugated carbon dots exhibited antibacterial activity against methicillin-resistant *S. aureus* and multidrug-resistant *E. coli*, with no associated cytotoxicity[244]. However, certain limitations remain in the research on carbon dots. Most antibacterial carbon dots that have reexhibit lower efficacy compared to conventional antibiotics, limiting their potential as effective replacements. Furthermore, the risk of AMR induced by exposure to carbon dots has not yet been sufficiently analysed[245].

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4.7 Dendrimers

Dendrimers are synthetic polymers consisting of multiple monomers branching from a central core. This greatly increases the number of ligand binding sites available, allowing for functionalization with multiple copies of a drug. The distribution and *in vivo* properties of the dendrimers can be controlled by modifying the size and structure of the polymer[246]. Due to their dendritic structures, these NPs exhibit a high degree of resistance to enzymatic hydrolysis and proteolytic degradation, which can otherwise degrade their monomeric constitutents. This structural advantage contributes to their enhanced stability *in vivo*, thereby improving their potential for biomedical applications[247]. Dendrimers act through the depolarization and lysis of the membranes of bacterial cells. The positively charged functional groups of dendrimers interact with negatively charged components of the membranes, and on contact, the dendrimers are fragmented. The fragments are inserted into the membrane, where disruption occurs. In addition, the dendrimers interfere with metabolic processes, including glycolysis, by inhibiting the synthesis of key proteins[248]. Depending on the design, structure and functional groups used, dendrimers can show both bactericidal and bacteriostatic activity.

Amine-terminating poly(amidoamine) dendrimers inhibited the growth of *P. aeruginosa* at very low concentrations (MIC 1.5 μg/mL), and *S. aureus* at higher concentrations (MIC 20.8 μg/mL) These dendrimers were reported to be toxic to human corneal epithelial cells, however coating them with PEG significantly reduced the toxicity[249]. A study of peptide dendrimers 92functionalised with fatty acids showed that dendrimers had increased activity against multiple-drug-resistant *E. coli*, *P. aeruginosa, A. baumannii* and methicillin-resistant *S. aureus*. Cytotoxicity assays of these dendrimers showed compatibility with multiple mammalian and human cell lines[250]. Another study of organometallic dendrimers reported antimicrobial activity against methicillin-resistant *S. aureus*, *S. warnerii,* and vancomycin-resistant *E. faecium*; but did not have any activity against *C. albicans*, *P. aeruginosa* and *P. vulgaris*[251]. Dendrimers are also used as drug delivery vehicles. Sulfamethoxazole delivered using poly(amidoamine) dendrimers was found to have greater solubility, a slight improvement of activity against *E. coli*, and sustained release over a longer time period[252]. The use of poly(amidoamine) dendrimers for erythromycin and tobramycin showed improved solubility but no significant increase in antimicrobial activity against multiple bacterial strains, including *S. aureus*, *P. aeruginosa* and *E. coli*[253]. Several studies

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have also demonstrated that dendrimers possess the ability to inhibit and disrupt biofilms formed by a range of bacterial and fungal species, including *S. aureus* and *P. aeruginosa*[254].

Cytotoxicity assays of different dendrimers show high variation in the level of toxicity towards various human cell lines. Multiple studies have reported hemolysis, neurological toxicity, and toxicity against renal and digestive organs. In addition, the consistency of dendrimer synthesis is difficult to achieve. Due to this, the current applications of dendrimers in medical fields are limited[255].

4.8 Hydrogels

Hydrogels are three-dimensional hydrophilic polymers that typically contain or can absorb large quantities of water[256]. Hydrogel NPs are advantageous as antibiotic carriers as they are biocompatible and biodegradable, making them less prone to cytotoxicity. Most hydrogels release drugs through passive diffusion and hence sustain the antibiotic presence in the body. The rate of diffusion can be controlled by the proportion of cross-linkage of the NPs. Hydrogels can also be modified to release the drugs only on stimulation. Stimulation methods include temperature, light, sound, electromagnetic fields and pressure[257].

Lee et al. developed antimicrobial hydrogels by combining an ABA-type triblock copolymer - PEG flanked by vitamin E-functionalized polycarbonate blocks - with a biocidal cationic polycarbonate also containing vitamin E. Both components were synthesized *via* ring-opening polymerization of functionalized cyclic carbonate monomers. Hydrogel formation relies on hydrophobic interactions between vitamin E moieties, enabling broad-spectrum antimicrobial activity and effective biofilm dispersion. The hydrogels demonstrated over 99.9% killing efficiency against *S. aureus*, *E. coli*, and *C. albicans* upon contact, showcasing potent broad-spectrum antimicrobial activity (**Figure 11**)[258]. Ciprofloxacin-loaded hydrogels were demonstrated to have sustained release of antibiotics against methicillin-resistant *S. aureus*, with a notable increase in antimicrobial activity as well[259]. Amikacin delivered using an alginate-derived hydrogel possessed significantly increased activity against *E.coli*, *S. epidermidis*, *S. aureus*, and *P. aeruginosa* at only 1.43% amikacin content, with a notable decrease in MIC. Assays of these hydrogels performed in mice also showed no cytotoxicity[260]. Antibiotics can also be released by hydrogel degradation, which results in a burst release. This reduces the persistence of the antibiotic. To avoid this, studies suggest using a two-step release mechanism, where the antibiotic is conjugated with another nanoparticle before loading, which restores the persistence

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of the drug[261]. Despite the low toxicity, multiple studies have reported the generation of immune responses against hydrogels, particularly those synthesized from biomaterials or functionalized with PEG. Hydrogels are also subject to high batch variation during synthesis, due to which they are considered unreliable for commercial production[262]. **Table 1** presents a comprehensive overview of the antimicrobial mechanisms exhibited by different types of nanoparticle materials against various pathogenic microorganisms. The table highlights the diversity of nanomaterials, such as metal-based, metal oxide, carbon-based, and polymeric NPs, along with their mode of action, including ROS generation, membrane disruption, ion release, biofilm inhibition, and intracellular targeting. These mechanisms collectively contribute to the broad-spectrum efficacy of nanomaterials and underline their potential as alternative or complementary agents to conventional antibiotics in combating AMR.

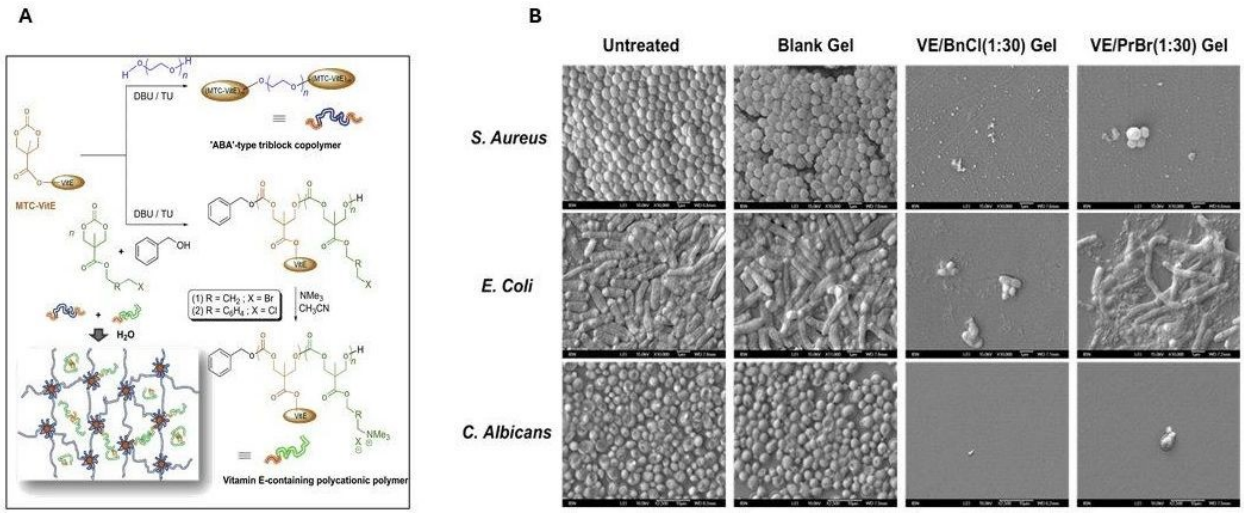

Figure 11: (A) Synthesis of '(MTC-VE)□-PEG-(MTC-VE)□' and vitamin E-based cationic polymers, along with a schematic representation of their incorporation into the hydrogel system; and **(B)** SEM images of biofilms exposed to polycation-loaded $(MTC\text{-}VE)_{1.25}$-PEG(20k)-$(MTC\text{-}VE)_{1.25}$ hydrogels (Reproduced with permission from Ref.[258], @ 2012, Elsevier).

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Table 1: Summary of antimicrobial activity mechanisms of various nanoparticle materials against target pathogens

Nanomaterial Category	Nanomaterial	Target Pathogen	Mechanism of Action	Ref.
Polymeric NPs	CS	*Propionibacterium acnes*	Inhibition of cytokine IL-12p40 in human monocytes and IL-6 in keratinocytes	[102]
		Gram-negative bacteria	Disruption of the integrity of the outer membrane under acidic conditions	[103]
		Aspergillus flavus and *A. parasiticus*	Suppression of aflatoxin production	[263]
		Bacteriophages	Inactivation of mature phage particles and inhibition of phage reproduction	[264]
Metal Ion NPs	ZnO	*S. aureus*	Production of Reactive Oxygen Species and disruption of cellular membranes	[265]
		Klebsiella pneumoniae	Rupturing of the cell membrane and suppression of metabolic activity by binding to intracellular material	[266]
		E. coli	Damage to the membrane wall	[267]
		Salmonella typhimurium	Lysis of the cell membrane	[160]

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		A. pleuropneumonia, *Salmonella typhimurium* and *Haemophilus parasuis*	Weak inhibition of bacterial growth and prevention of biofilm formation	[268]
	Ag	*E. coli*	Formation of pits in the membrane, inhibition of DNA replication, and denaturation of proteins	[171]
		E. coli and *S. aureus*	Generation of free radical species	[97]
		Pseudomonas aeruginosa	Disruption of biofilms and inhibition of biofilm formation	[97]
	Cu	Gram-negative bacteria	Formation of pits in the cellular wall, generation of reactive oxygen species, degradation of DNA, oxidation of metabolic proteins	[184], [269]
		E. coli	Disruption of the cell wall, leading to degradation of the cytoplasm	[183]
	Au	*S. aureus, K. pneumoniae, E. coli and B. subtilis*	Inhibition of ATP synthetase, degradation of sulphur-containing proteins and blocking of tRNA binding sites	[191], [192]

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	Titanium	Gram-negative and Gram-positive bacteria	Increased permeability of the membrane, causing leakage of cellular components	[200]
		E. coli, P. aeruginosa, S. aureus, *M. luteus*	Disruption of the cell membrane and plasmolysis	[200]
	Magnetic	*S. aureus*, *A. viridians*, and *E. columbae*	Inhibition of biofilm formation	[270]
		E. coli and *S. aureus*	Inhibition of protein synthesis and disruption of metabolic processes, including respiration	[205], [206]
	Cobalt	*S. aureus*	Disruption of the cell membrane, interruption of DNA replication, and inhibition of proteins	[214], [271]
		E. coli	Generation of Reactive Oxygen Species	[272]
	Selenium	*E. coli* and *S. aureus*	Penetration of the outer membrane, modification of biosynthetic enzymes	[218]
		Candida albicans	Inhibition of preformed biofilms	[219]
	Cadmium Oxide	*S. aureus*, *K. pneumoniae*, *A. baumannii, E. coli*, *B. subtilis* and *P. aeruginosa*	Increased permeability of the membrane, generation of reactive oxygen species, degradation of essential proteins and DNA	[223], [225]

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Other Nanomaterials	Carbon Dots	Gram-negative bacteria	Blocking the generation of pro-inflammatory cytokines	[235]
		E. coli and *S. aureus*	Increased permeability of the cell and decreased integrity of the plasma membrane	[273]
		S. aureus, *Bacillus sp.*, *E. coli*	Disruption of secondary structures of DNA and RNA	[274]
		Multi-drug-resistant *Enterococcus faecium*, *E. faecalis, S. aureus* (MRSA)	Generation of Reactive Oxygen Species and degradation of the bacterial cell wall	[243], [244]
	Dendrimers	*P. aeruginosa*	Formation of holes in the lipid bilayer	[249], [250]
		Multi-drug-resistant *S. aureus, E. faecium* and *A. baumannii*	Disruption of the cell membrane and leakage of cellular contents	[250], [251]

Further, **Table 2** provides a comparative summary of the bacteriostatic and bactericidal effects exhibited by various nanomaterials commonly explored for antimicrobial applications. Several nanomaterials, such as CS, AgNPs, ZnO NPs, and dendrimers, demonstrate both bacteriostatic (inhibition of bacterial growth) and bactericidal (bacterial killing) properties, depending on their physicochemical characteristics, concentration, and the type of microorganism targeted. In contrast, materials like AuNPs and carbon dots primarily exhibit bacteriostatic behavior, often requiring functionalization or combination with other agents to achieve bactericidal action. This distinction is critical for selecting appropriate nanomaterials for specific clinical or environmental

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applications, especially when considering the severity of infection, pathogen type, and potential resistance mechanisms.

Table 2: Bacteriostatic and bactericidal effects of nanomaterials

Nanomaterial Type	Effect	
CS	Bacteriostatic	Bactericidal
Ag NPs	Bacteriostatic	Bactericidal
Au NPs	Bacteriostatic	-
ZnO NPs	Bacteriostatic	Bactericidal
Dendrimers	Bacteriostatic	Bactericidal
Carbon Dots	Bacteriostatic	-

5. LIMITATIONS OF NANOTECHNOLOGY-BASED APPROACHES

Despite the promising potential of nanotechnology in addressing AMR, several limitations hinder its clinical translation and widespread application. One major concern is the toxicity and biocompatibility of certain NPs, especially those composed of heavy metals such as Ag, Cu, or ZnO. These materials, while effective against microbes, may induce oxidative stress, inflammation, or cytotoxic effects in host tissues. Additionally, long-term safety data are often lacking, raising concerns about bioaccumulation and unintended ecological impacts following environmental release. Another challenge lies in the scalability and reproducibility of nanoparticle synthesis, which can result in batch-to-batch variations affecting consistency in therapeutic outcomes[275]. The complexity of regulatory approval processes for nanomaterials also slows down clinical implementation, as existing frameworks may not fully accommodate the unique properties of nanomedicines. Moreover, bacteria may eventually develop resistance mechanisms even against nanoscale antimicrobials, particularly if these systems are overused or misapplied. Finally, cost-effectiveness and integration into existing healthcare systems remain significant hurdles, especially in low-resource settings where AMR is most prevalent. Addressing these limitations through rigorous toxicological studies, standardization of nanoparticle synthesis, and multidisciplinary regulatory efforts will be essential for realizing the full potential of nanotechnology in AMR mitigation.

5.1 Nanoparticle Cytotoxicity

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A significant limitation of many cytotoxicity assays is that they are conducted in vitro, using cultured cell lines. While this approach provides insights into nanoparticle effects on individual cells, it fails to account for the interactions between different cell types within organs, which are crucial for accurately reflecting in vivo effects on the host system. Despite this limitation, in vitro toxicity assays remain essential for assessing the potential risks of NPs[276]. The toxicity of NPs is influenced by various characteristics, including size, shape, and charge, all of which depend heavily on the synthesis process, where consistency is often not guaranteed[277]. Due to their small size, NPs can easily penetrate host cells and organs, demonstrating the ability to infiltrate the liver, spleen, and even the blood-brain barrier. When accumulated in high concentrations, they can generate reactive oxygen species that may cause significant damage to these organs[278]. Additionally, NPs have been associated with hemolysis and thrombogenicity in blood cells[279]. Most nanoparticle therapies are delivered to their site of action through bloodstream. The liver, functioning as a filter for blood impurities, often becomes a common site for nanoparticle accumulation. While studies have shown that Kupffer cells and sinusoidal epithelial cells can remove these particles, this process is relatively slow. Prolonged exposure to NPs can negatively affect the metabolic pathways of liver cells, with consequences that may last for several months. Furthermore, the neutralization of NPs by Kupffer cells can trigger the synthesis of pro-inflammatory cytokines, leading to liver inflammation[280].

Another important concern is immunotoxicity. NPs are frequently functionalized with biomolecules such as proteins, oligosaccharides, and immunoglobulins to enhance drug delivery specificity. However, these ligands can provoke an immunogenic response in the host. Additionally, opsonization of the NPs by plasma proteins may activate the immune system, resulting in further inflammation of body tissues[281].

5.2 Nanoparticle Resistance

As research on AMR progresses, a growing concern is the potential development of resistance to the alternative treatment methods currently under investigation. NPs, particularly metal ion NPs, typically exhibit antimicrobial activity by disrupting various aspects of bacterial cells, including cell membranes, protein synthesis, electron transport, and DNA transcription. Consequently, the emergence of resistance to NPs requires multiple mutations within the bacterial cell, making it less likely than traditional AMR. Furthermore, the diverse targeting mechanisms of NPs diminish the risk of simultaneous resistance to multiple nanoparticle types[282]. Despite this, several studies have

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reported the development of resistance to NPs[283]. For instance, genomic analysis of *E. coli* exposed to Ag NPs revealed that three mutations conferring resistance spread throughout the population within 200 generations[284]. Common mechanisms of resistance include mutations that alter membrane permeability and changes in biofilm composition[283]. Additionally, adjustments in efflux pumps to expel metal ion NPs have been observed[282]. Notably, these resistance adaptations can be transferred between bacterial cells through horizontal gene transfer.

Nanotechnology holds significant promise in addressing AMR, with numerous studies highlighting its potential in combating resistant pathogens. However, translating laboratory successes into clinically viable systemic treatments requires substantial further research and investment. One of the primary challenges in the in vivo application of NPs is their complex interaction with the human body. When administered intravenously, NPs must overcome biological barriers, including clearance by phagocytic immune cells. NPs smaller than approximately 10 nm are rapidly filtered out by the kidneys, whereas those larger than 200 nm are prone to activating the complement system, potentially triggering undesirable immune responses. To improve their pharmacokinetic properties, polyethylene glycol (PEG) is frequently used to modify NPs, a process known as PEGylation. This "stealth" coating enhances solubility, extends circulation time, and reduces recognition by the immune system. However, pre-existing anti-PEG antibodies, possibly acquired through environmental or non-medical exposure, may compromise the effectiveness of PEGylated formulations. Additionally, the stability of NPs in the bloodstream is influenced by their composition. Lipid- and polymer-based NPs, for example, are more prone to aggregation and colloidal instability. To address this, surface modifications such as cross-linking with polymers and PEGylation are commonly employed to enhance stability and improve therapeutic performance. Research into nanoparticle resistance is still ongoing, and the conditions leading to its development are not yet fully understood. Identifying the factors that contribute to this resistance and exploring potential strategies to mitigate it are crucial for preserving the efficacy of NPs as antimicrobial agents. **Fig. 12** outlines key limitations of nanomaterial-based strategies, including cytotoxic effects on host cells, ecological impact, suboptimal encapsulation efficiency, high costs of synthesis and characterization, and the emergence of resistance to nanomaterials. Additional concerns involve nanoparticle aggregation and bioaccumulation, batch-to-batch variability in formulation, and the lack of comprehensive long-term safety data. These factors

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hinder the widespread and sustainable application of nanotechnology in clinical and environmental settings.

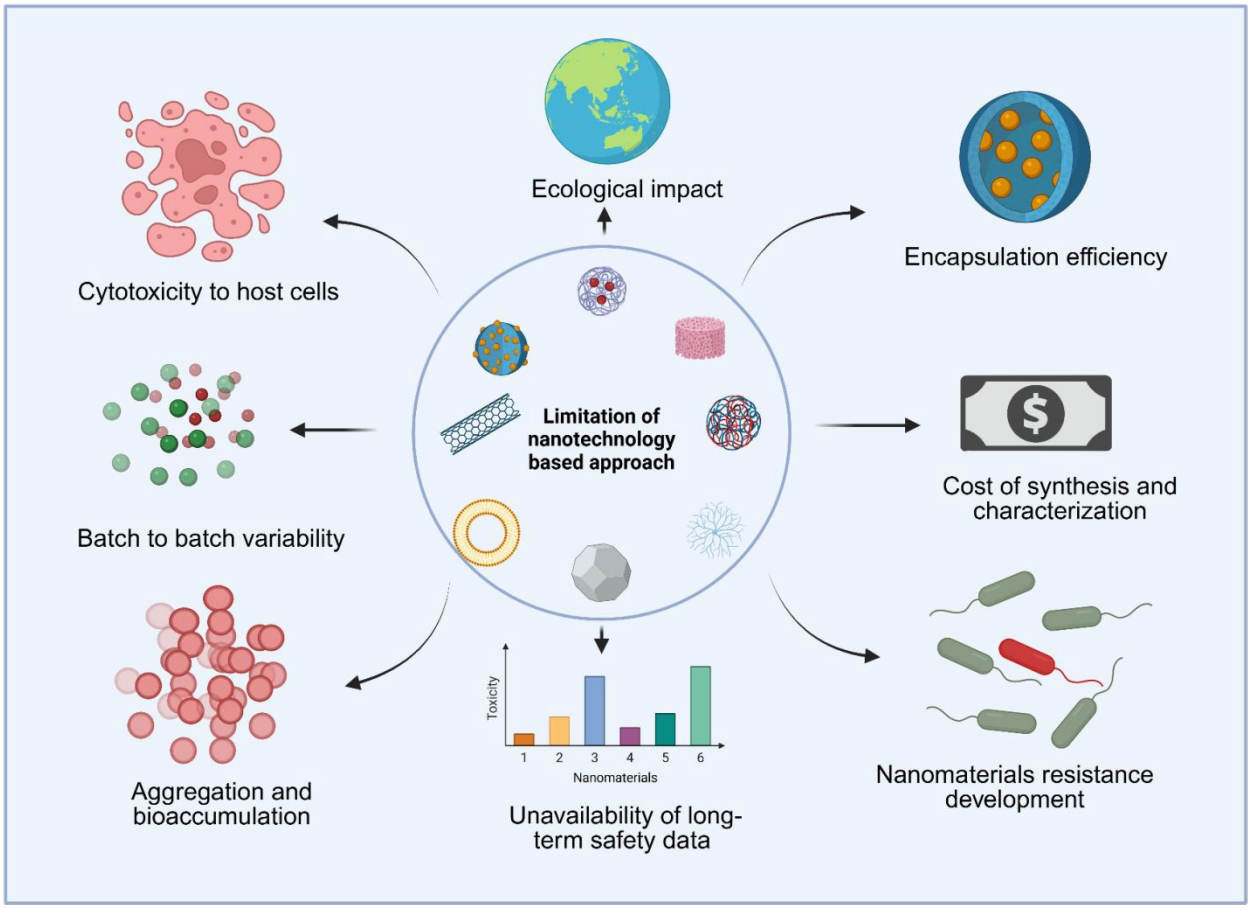

Figure 12: Limitations of nanotechnology-based approaches for drug delivery against antimicrobial resistant pathogens.

5.3 Toxicological and ecological risks within a One Health framework

The use of NPs to combat AMR within a One Health framework brings significant promise but also raises critical concerns regarding their toxicological impacts and ecological risks across human, animal, and environmental domains.

Potential Toxicological Impacts: Certain NPs, such as AgNPs, have been shown to induce adverse biological effects in mammalian systems. For example, AgNPs can impair cellular antioxidants, leading to increased ROS accumulation, mitochondrial and DNA damage, lipid peroxidation, apoptosis, and ultimately, impaired reproductive functions such as reduced sperm quality and hormone production. These effects are especially concerning given the widespread use

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and environmental release of AgNPs, which can result in unintended human exposure through water, food, or direct contact.

NPs like TiO_2 and ZnO exhibits pronounced toxicity in aquatic organisms. For instance, nanosized TiO_2 can adhere to the surface of aquatic invertebrates such as Daphnia magna, disrupting physiological processes like molting and leading to high mortality rates. This surface coating effect, combined with the persistent presence of NPs in water, can have widespread negative impacts on aquatic health and biodiversity. Similarly, ZnO NPs can undergo transformation into sewage treatment environments, forming new nanoparticle species (e.g., ZnS NPs) with altered stability and dissolution rates, potentially increasing their toxicity and persistence in aquatic ecosystems. Environmental factors such as pH, ionic strength, and the presence of other chemicals can alter the form, stability, and toxicity of NPs. These transformations may enhance their bioavailability and facilitate bioaccumulation in food webs, posing risks not only to aquatic organisms but also to higher trophic levels, including humans.

Ecological Risks in One Health Ecosystems: The accumulation and persistence of NPs in the environment can degrade ecosystem services such as water quality, fisheries, and biodiversity. For example, NPs can disrupt the reproductive, developmental, and immune functions of aquatic species, leading to population declines and altered ecosystem dynamics. Further, NPs released into the environment can be transported across air, water, and soil, leading to exposure to animals and humans far from the original point of release. This mobility increases the risk of cross-species contamination and highlights the interconnectedness emphasized by the One Health approach.

Despite increasing evidence of toxicity, there is still a lack of comprehensive data on the long-term fate, transport, and chronic effects of NPs in complex real-world ecosystems. Existing toxicity assessments often rely on short-term laboratory tests, which may not capture the full spectrum of ecological risks. While nanotechnologies offer innovative solutions for AMR, their deployment must be accompanied by rigorous assessment of toxicological and ecological risks. This includes understanding nanoparticle transformations, potential for bioaccumulation, and impacts on ecosystem health, as well as developing regulatory frameworks that reflect the interconnected realities of One Health ecosystems.

5.4 Scalability and Commercialization Potential of Nanotechnology-Based Approaches for AMR Management

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The scalability and commercialization potential of nanotechnology-based approaches for AMR management are promising yet complex, reflecting both remarkable advancements and persistent challenges. Nanomaterials such as metal and metal oxide NPs, liposomes, dendrimers, and polymeric carriers offer unique physicochemical properties that enable targeted antimicrobial delivery, enhanced efficacy, and the ability to bypass traditional resistance mechanisms. These attributes have driven significant interest from both academic and industrial sectors in developing nano-enabled therapeutics, diagnostics, and antimicrobial coatings for clinical, veterinary, and environmental applications. However, translating laboratory-scale innovations into commercially viable products requires overcoming several critical hurdles. First, the reproducible large-scale synthesis of nanomaterials with consistent quality and functionality remains a major bottleneck. Variability in nanoparticle size, shape, surface chemistry, and batch-to-batch consistency can impact both efficacy and safety, complicating industrial production and regulatory approval processes. Moreover, the cost-effectiveness of manufacturing and the scalability of green or sustainable synthesis methods such as plant-derived or biogenic NPs are under active investigation, as these approaches may reduce environmental impact and facilitate broader adoption.

Commercialization also hinges on robust toxicological and biocompatibility assessments, which are essential for gaining regulatory approval and market acceptance. Current regulatory frameworks for nanomedicines and nano-antimicrobials are still evolving, with a lack of harmonized guidelines across jurisdictions. This regulatory uncertainty can delay product development and increase commercialization risks. Additionally, concerns about long-term safety, environmental persistence, and potential ecological risks of NPs release must be addressed through comprehensive risk assessments and lifecycle analyses. Despite these challenges, several nano-enabled antimicrobial products such as wound dressings, coatings for medical devices, and food packaging have reached the market, demonstrating the feasibility of commercial translation in specific sectors. Ongoing interdisciplinary collaboration among researchers, industry stakeholders, and policymakers is critical to optimize production processes, standardize safety evaluations, and establish clear regulatory pathways. With continued investment in scalable manufacturing technologies and regulatory science, nanotechnology-based solutions hold substantial potential to transform AMR management across the One Health spectrum.

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6. SUMMARY AND FUTURE PROSPECTS

The rise of AMR is a concerning trend that threatens to undermine the progress made by antimicrobial agents in the biomedical field. Among various strategies proposed to address this issue, research on nanotechnology-based approaches has shown significant advancement in recent decades. NPs have been shown to significantly enhance the efficacy of antimicrobial drugs by enabling controlled, sustained release and circumventing conventional resistance mechanisms. Through surface modification with functional groups and ligands, they can be engineered for increased specificity, minimizing the risk of side effects. Furthermore, many NPs possess intrinsic antimicrobial activity that is unaffected by traditional resistance mechanisms, broadening their therapeutic applications. Materials such as CS, PLGA, Au, and ZnO have demonstrated favorable biocompatibility through extensive studies and trials. In contrast, materials such as Cu and titanium oxide have exhibited potential cytotoxic effects, and some, including silicon and cadmium, require further evaluation due to insufficient data. Clinical adoption of nanoparticle-based therapeutics is already underway, with several PLGA and liposome-based NPs receiving approval for specific clinical applications. Additionally, certain NPs, such as CS and iron oxide, have been successfully employed in other biomedical domains, including magnetic resonance imaging. The antimicrobial functionality and enhanced drug delivery capabilities make NPs a potent tool in combating the reappearing threat of microbial infections.

Increasing concerns regarding the cytotoxicity of NPs have prompted extensive research into strategies to mitigate associated risks. One prominent approach involves surface modification through the application of polymeric coatings. This significantly reduces the rate of ion release, allowing for less aggressive delivery. Several coating materials have demonstrated potential, particularly organic polymers such as CS, saccharides, and polyphenols[285]. Among these, polyethylene glycol (PEG) has emerged as particularly effective, with numerous studies reporting its ability to significantly reduce protein adsorption on nanoparticle surfaces[286]. As a result, PEGylated NPs are commonly employed in clinically approved drug formulations. Similarly, functionalization of nanoparticle surfaces with specific ligands can be performed to optimize interactions with the biological environment. This approach enhances delivery specificity and minimizes off-target interactions with non-targeted cells. Such modifications can significantly reduce cytotoxic effects on critical organs, including the lungs, liver, and kidneys[287]. Another emerging strategy is green synthesis, which utilizes biological processes, such as those involving

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plant extracts, bacteria, or fungi, for nanoparticle production. NPs generated through this approach are typically surface-functionalized and exhibit high biocompatibility. However, a major limitation lies in the difficulty of obtaining homogenous biological materials, which poses challenges in achieving reproducible and uniform nanoparticle synthesis[285].

Although clinical trials involving NPs have begun to show promise, regulations governing their use in medicine remain inadequate. The US Food and Drug Administration, the European Medicines Agency, and other government organizations have introduced restrictions on research and trials related to NPs, but these regulations are limited to national contexts. There is currently no international consensus on what constitutes a nanomaterial-based drug, with most agencies operating with different definitions. Similarly, the criteria for manufacture, handling, biocompatibility and cytotoxicity remain undefined[288]. This regulatory void hinders progress in the field, deterring investors and pharmaceutical companies from pursuing research in this area[132]. Additionally, the lack of clarity slows the approval of nanoparticle-based drugs; since 2016, only 13 drugs have been approved by the FDA, most of which are liposomal formulations[289]. This underscores the urgent need for international agreements and regulations concerning the study and clinical use of NPs, as without them, the field's growth will stagnate. **Table 3** summarizes a selection of FDA-approved nanoparticle-based formulations currently used in clinical medicine, along with the developmental status of emerging nanoparticle therapeutics specifically targeting AMR. This includes liposomes, polymeric NPs, and metallic nanocarriers approved for drug delivery, cancer therapy, and infectious disease treatment. The table also outlines ongoing research and clinical trials exploring nanoparticle-enabled strategies to combat multidrug-resistant pathogens, showcasing their potential to overcome the limitations of conventional antibiotics through enhanced delivery, stability, and targeted action.

Table 3: Summary of various FDA-Approved NPs for medical purposes, and development status of nanoparticle drugs for AMR[290,291].

Disease	FDA-Approved Nanoparticle Materials
Acromegaly	Polymeric NP (PEG)
Amyloidosis	Lipid NP
Anemia	Polymeric NP (PEG)

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Arthritis	PLGA Hydrogel, Polymeric NP (PEG)
Carcinoma	Metallic Oxide NP
Chronic Kidney Disease	Iron-based NP
COVID-19	Liposomal NP
Fungal Infections	Liposomal NP
Glioblastoma	Iron Oxide NP
Hemophilia	Polymeric NP
Hepatitis	Polymeric NP (PEG)
Leukemia	Liposomal NP, Polymeric NP (PEG)
MAC Lung Disease	Liposomal NP
Meningitis	Liposomal NP
Multiple Sclerosis	Polymeric NP (PEG)
Opioid Use Disorder	PLGA NP
Pancreatic Cancer	Liposomal NP
Prostate Cancer	Polymeric NP (PLGH)
Sarcoma	Liposomal NP
Severe Combined Immunodeficiency Disease	Polymeric NP (PEG)
Shingles	Liposomal NP
Nanoparticle Drugs for AMR (sourced from clinicaltrials.gov)	
Nanoparticle Drug	**Development Status**
PLGA-Hydrogel NPs for *E. faecalis*	Completed Clinical Trials
Ciprfloxacin-loaded PLGA-Chitson NPs for *E. faecalis*	Completed Clinical Trials
TiO_2 NPs for *C. albicans*	Completed Clinical Trials
Miconazol-loaded CS NPs for *C. albicans*	Completed Clinical Trials
Ag NPs for antifungal activity	Clinical Trials

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ZnO for antifungal activity	Completed Clinical Trials

A further challenge regarding the use of NPs in medicine is their mass production for commercial use. As previously mentioned, the conditions under which NPs are synthesized significantly influence their physicochemical characteristics, such as surface charge and size, which in turn affect their antimicrobial activity[277]. Standardization of manufacturing processes is essential for the proper commercialization of nanoparticle-based drugs. Furthermore, before NPs can be integrated into clinical practice, their cytotoxicity must be thoroughly evaluated *in vitro*, particularly under varying environmental conditions. While several studies have reported the cytotoxicity of various NPs, especially metal ion NPs, a comprehensive understanding of the mechanisms of toxicity and the cytotoxic concentrations is needed. Additionally, the interactions of NPs with other antimicrobial agents, the potential effects of these interactions, and the consequences of nanoparticle biodegradation require further investigation.

Ongoing research is also exploring a range of nanoparticle-based technologies aimed at improving antimicrobial drug delivery. One such innovation involves the development of nanozymes, which are nanomaterials that mimic the structure and catalytic functions of natural enzymes[292]. Several natural enzymes, such as lysozymes, oxidases, and peroxidases, possess antimicrobial properties through catalyzing the hydrolysis of key structural and metabolic components of pathogenic cells. By replicating these enzymes, nanozymes have demonstrated promising antibacterial activity. In addition, nanozymes have enhanced stability and prolonged functionality under physiological conditions, giving an advantage over their natural counterparts[293]. Another technology currently under investigation is the use of stimuli-responsive nanocarriers. These are engineered NPs designed to release the loaded molecule only in response to specific stimuli, allowing for site-specific and highly targeted drug delivery. A wide range of stimuli have been explored, including light, heat, magnetic fields, ultrasound, and the presence of ions. This approach not only minimizes off-target effects but also facilitates precise control over drug dosage and release kinetics[294]. These technologies hold significant promises for advancing the clinical management of microbial infections through improved efficacy, specificity, and safety profiles. In conclusion, while nanotechnology offers promising solutions to combat AMR, the development of robust regulations, standardization of manufacturing processes, and comprehensive toxicity assessments are essential to ensure the safe and effective integration of NPs into clinical practice.

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7. CONCLUSION

AMR stands as one of the most urgent global health challenges of the 21st century, threatening the efficacy of modern medicine and the sustainability of ecosystems. This review has highlighted the multifactorial nature of AMR, emphasizing the interconnectedness of human, animal, and environmental health, an approach central to the One Health paradigm. By synthesizing current knowledge on the mechanisms of resistance, traditional and emerging therapeutic strategies, and the unique contributions of nanotechnology, this review underscores both the promise and complexity of tackling AMR in a holistic manner. Nanotechnology based interventions have emerged as a transformative frontier in the fight against drug-resistant pathogens. Diverse platforms ranging from CS and PLGA NPs to metallic NPs, dendrimers, and hydrogels, demonstrate potent antimicrobial activity, often through mechanisms distinct from conventional antibiotics. These include membrane disruption, ROS generation, and targeted delivery, offering new hope for overcoming established resistance pathways and enhancing the efficacy of existing antimicrobials. However, the translation of nanotechnologies from bench to bedside and into broader One Health applications is not without significant challenges. This review has critically examined the limitations associated with nanoparticle use, including cytotoxicity, the potential for nanoparticle-induced resistance, and the still-underexplored toxicological and ecological risks. The persistence and bioaccumulation of NPs in the environment, their effects on non-target organisms, and the long-term consequences for ecosystem health remain areas of active concern and ongoing research.

Furthermore, issues related to scalability, reproducibility, biocompatibility, and regulatory oversight present formidable barriers to clinical and field deployment. The absence of harmonized guidelines and robust long-term safety data underscores the need for interdisciplinary collaboration among scientists, clinicians, regulatory bodies, and policymakers. Looking forward, the integration of emerging nanotechnology platforms such as stimuli-responsive systems, hybrid nanosystem, and bioengineered nanozymes offer exciting avenues for innovation. Yet, their successful implementation will depend on addressing current knowledge gaps, particularly in clinical validation, environmental safety, and regulatory clarity. In conclusion, a One Health nanotechnologies approach provides a strategic and integrative framework for addressing AMR. By embracing the complexity of AMR transmission across human, animal, and environmental

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domains, and by fostering responsible innovation in nanotechnology, the global community can move closer to sustainable, effective solutions. Continued research, cross-sectoral collaboration, and proactive risk assessment will be essential to realize the full potential of nanotechnologies in safeguarding public health and ecosystem integrity against the escalating threat of AMR.

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Conflict of Interests

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Data Availability Statement

Data shall be made available upon genuine request to the authors.