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Antibiotic Resistance Risk and Ecosystem

Antibiyotik Rezistans Riski ve Ekosistem

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ABSTRACT

Antibiotics are among the most prescribed drugs in human medicine. However, antibiotic resistance is a continuing global human health problem. Ecosystems and clinical practices play equally important roles in the emergence, evolution, and spread of antibiotic resistance. High doses and inappropriate antibiotic use result in large amounts of antibiotics being released into the ecosystem, leading to the selection of large numbers of resistant bacteria and the spread of antibiotic resistance genes (ARGs). Accelerated selection of antibiotic-resistant strains and increasing numbers of antibiotic-resistant bacteria (ARB) result in increased infection-related patient mortality rates. Hosts of environmental resistomes are largely restricted to the core of the environmental microbiome. Because of the phylogenetic barriers to horizontal transfer of ARGs, gene transfer occurs more frequently within species than between species. Horizontal gene transfer (HGT) drives the evolution of untreatable “superbugs” by concentrating ARGs within individual cells. Decreasing the colonization capacity of exogenous ARB, increasing human resistance to colonization, reducing horizontal transmission of ARG, and reversing antibiotic resistance in ARB are the most important strategies to combat antibiotic resistance by preventing transmission of ARB and ARG to humans.

Keywords: Antibiotic resistance, ecosystem, horizontal gene transfer, mobile genetic element, marine organism

Öz

Antibiyotikler, tıbbi uygulamalarda en çok reçete edilen ilaç gruplarından biridir. Ancak antibiyotik direnci, halen küresel bir insan sağlığı sorunudur. Ekosistemler ve klinik uygulamalar, antibiyotik direncinin ortaya çıkmasında, evriminde ve yayılmasında eşit derecede önemli rol oynar. Yüksek dozlarda ve uygunsuz antibiyotik kullanımı, ekosisteme büyük miktarda antibiyotik salınımına ve çok miktarda dirençli bakterinin seçilimine ve antibiyotik direnç genlerinin (ARG) yayılmasına yol açar. Antibiyotik dirençli suşların hızla seçilimi ve artan sayıda antibiyotik dirençli bakteri (ARB), enfeksiyona bağlı hasta ölüm oranlarının artmasına neden olur. Çevresel direnç genlerinin konakçıları büyük ölçüde çevresel mikrobiyom ile sınırlıdır. ARG'lerin yatay transferine yönelik filogenetik engeller nedeniyle, gen transferi türler arasında değil, türler içinde daha sık gerçekleşir. Yatay gen transferi (HGT), ARG'lerini aynı hücrede bir araya toplayarak tedavi edilemeyen “süper mikropların” evrimini yönlendirir. Ekzojen ARB'lerin kolonizasyon kapasitesini azaltmak, insanlarda kolonizasyona karşı direnci artırmak, ARG'nin yatay bulaşmasını azaltmak ve ARB antibiyotik direncini tersine çevirmek, ARB ve ARG'nin insanlara bulaşmasının önlenmesinde ve antibiyotik direnciyle mücadelede en önemli stratejilerdir.

Anahtar Sözcükler: Antibiyotik direnci, ekosistem, yatay gen transferi, mobil genetik element, deniz organizmaları

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INTRODUCTION

Antibiotics are among the most prescribed drugs in human medicine. However, global estimates suggest that only 50% of global antibiotic use is appropriate (1). In the United States of America, the Centers for Disease Control and Prevention (CDC) analysis of data from the National Ambulatory Medical Care Survey and the National Hospital Ambulatory Medical Care Survey indicate that 30% of the 154 million antibiotics prescribed each year are considered unnecessary (2). When the antibiotics are widely used, the reservoirs of resistant genes are prevalent, thus these tend to spread through ecological niches due to complex interactions between people in different environments (3). Antibiotic resistance is a continuing global human health problem. Controversially, the healthcare facilities contribute significantly to the emergence, evolution, and spread of antibiotic resistance, however also the ecosystems have an equally important role in its spread (4). Disposal of household medical waste and unused antibiotics in landfills or in wastewater poses a significant environmental and public health hazard. A significant portion of these drugs consists of stable compounds that are difficult to remove in wastewater treatment plants (WTPs) and that accumulate in the environment. Antibiotics such as ciprofloxacin and trimethoprim, as well as antineoplastic drugs such as cyclophosphamide, have been isolated from these waters (5). The contamination of hospital and city sewage wastewater with antibiotics and cytostatic drugs poses a major environmental threat (6). Cytostatic drugs, which inhibit DNA replication and are genotoxic to actively dividing cells of algae and aquatic plants, affect all proteins involved in the regulation of growth rate, the cell cycle, and redox status. Prolonged exposure of organisms to these toxins lead to unforeseen changes at the molecular level for the entire ecosystem (7-9). When considering the reuse of treated wastewater and biological solids for agricultural purposes, mutagenic potentials such as alteration in the

chromosomal aberration and micronucleus frequency are extremely important in terms of bacterial behavior as well as the extent of environmental pollution (10). While assessing the causal relationship between antibiotic resistance and clinical practice, numerous confounding factors exclusive to each drug and microorganism, like pathogen-pathogen and pathogen-host interactions, mutations, cross-resistance, resistance mechanisms, and cross-reservoir drivers, must be considered (2). Antimicrobial resistance is associated with an estimated 4.95 million deaths due to the bacterial infections and the substantial cost of this phenomenon to the global economy which may amount to US\$ 100 trillion until 2050 if no action is taken (11). The aim of this review is to discuss the “vicious cycle of environmental resistance” in which high doses of antibiotics released into the ecosystem during antibiotic administration lead to the selection of numerous resistant bacteria and the spread of antibiotic resistance genes (ARGs) (Figure 1). This comprehensive effort selected reports from a set of systematic research studies—performed with robust, validated methods and considering the significant and interdependent nature of the antibiotic resistance, encompassing human, animal, and environmental dimensions—to outline environmental antimicrobial-resistant (AMR) bacterial pollution and to discuss mechanisms of resistance development and their environmental properties. In this context, 98 articles were extracted from published studies, which were obtained by searching with the combined keywords “antibiotic resistance, ecosystem, horizontal gene transfer (HGT), and mobile genetic element”, and 66 of these were conducted in the last 10 years. Since combining antibiotics with nanoparticle (NP)-based carriers offers significant advantages in overcoming antibiotic resistance, which is a global problem today, a comprehensive clinical perspective is provided to encourage the integration of nanoantibiotics (nAbts) into clinical practice.

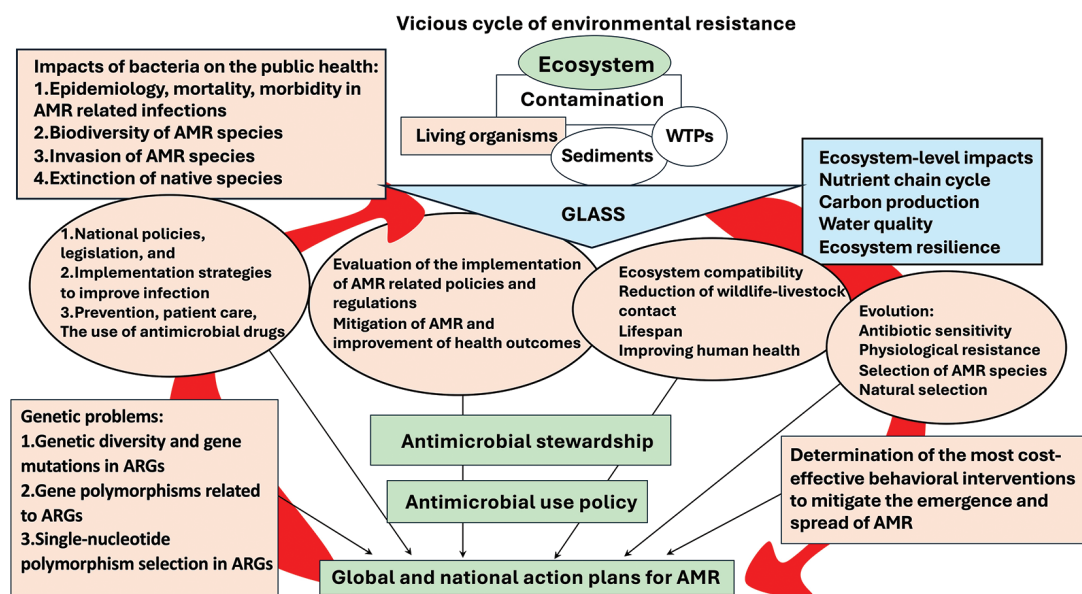


Figure 1. Vicious cycle of environmental resistance and the supporting factors of GLASS.

ARG: Antibiotic resistance genes, AMR: Antimicrobial resistance, GLASS: Global Antimicrobial resistance surveillance system - launched by World Health Organization in 2015, WTPs: Wastewater treatment plants.

Ecological Risk Assessment

Ecological risk assessment is based on the maximum measured concentration of pharmaceutical compounds in wastewater, expressed as a risk quotient (RQ), which is defined as the ratio of a point estimate of exposure [estimated environmental concentration (EEC) or predicted environmental concentration (PEC)] and a point estimate of effects (12). Compared with marine organisms, the maximum RQ values were 81,000 for ofloxacin and 440 for ciprofloxacin. These results indicate that antibiotics entering wastewater systems pose a high ecological risk due to improper wastewater treatment. This risk also means that waste pharmaceuticals can contaminate the food web and lead to antibiotic resistance in pathogenic bacteria (13). Twenty-two studies reporting drug concentrations in river and marine sediments have been published since 2000. A total of 62 drugs and their by-products were detected in marine precipitate at concentrations equivalent to 2,615,000 ng/g wet weight. Among these antibiotics, 26 were found to have the highest concentrations in the sediment (14). Fifty-nine papers published between 2002 and 2015 examined concentrations of 169 compounds in sludge from approximately 450 treatment systems, yielding data on 152 chemicals across 28 classes. Considering the environmental risk assessment of these compounds in soil, the most critical ones posing environmental risks in soil contaminated with sewage sludge were ciprofloxacin, ofloxacin and tetracycline (15). Fourteen studies reporting drug concentrations in shellfish measured tissue levels of 60 drugs and 7 by-products. Thirty-eight types of antibiotics constituted the most frequently reported contaminant class in these studies, followed by six types of antihypertensive agents. Ciprofloxacin and enrofloxacin were the most commonly reported antibiotics, each identified in at least four investigations. Only three reports reported the presence of erythromycin-based metabolites and its pharmaceutical transformation products (16,17). The effects of these pollutants were assessed in mussel tissues using biomarkers, such as activities of superoxide dismutase, catalase, glutathione reductase, and phase II glutathione-S-transferase (GST); lipid peroxidation levels (LPO), indicating oxidative stress; and acetylcholinesterase activity, associated with neurotoxicity. The results indicated potentially harmful impacts on species that may be sensitive to the continuous discharge of active pharmaceutical ingredients (APIs) into the ecosystem. Some compounds were found to significantly increase LPO levels in the liver, while others were found to increase GST levels in the gills. Indeed, APIs are commonly detected as environmental contaminants in surface waters due to incomplete and inappropriate wastewater treatment processes (18-20). Interestingly, however, some drugs were determined in marine organisms despite they could not be found in the sampled waters or sediments at concentrations below those expected to produce toxic effects (17).

Antibiotics and Their Ecological Effects

The use of antibiotics without a prescription or as a result of inappropriate prescribing leads to increased AMR, treatment failures, drug toxicity, and increased treatment costs (21). The World Health Organization (WHO) has identified AMR as one of the 10 most important threats to global health (22). A clear example of this occurred during the Coronavirus disease-2019 (COVID-19) pandemic in 2020. As an inevitable consequence of

the increase in inappropriate antibiotic usage during the COVID-19 pandemic, AMR rates have increased significantly (23). In parallel, the heavy contamination of sewage with antibiotics has accelerated the selection of antibiotic-resistant strains within the bacterial population. This increasing number of antibiotic-resistant bacteria (ARB) results in increased infection-related mortality rates of patients (24). These suggest that antibiotics play a significant role in drug contamination of environmental waste and in their ecological impacts. Thus, erythromycin-base has been detected at concentrations of 3.4 ng/g in San Francisco Bay, United States, and 0.7-14 ng/g in the Pearl River, China (25). Furthermore, in waters sampled in Southern China, 27 antibiotics, primarily quinolones (6.36-463 ng/L) and aminoglycosides (94.9-458 ng/L), were identified as posing ecological risks. Higher concentrations of antibiotics are observed in river water during the dry season than during the wet season. Organic matter and ammonia nitrogen in the sediment strongly influence the distribution of these antibiotics. Nevertheless, the levels of antibiotics detected in this region are not considered significant in terms of ecological risk (26). However, in the analysis of sediment samples from the Liao River basin in China, 19 different antibiotics were identified, with tetracyclines and macrolides being the predominant antibiotics in terms of concentration. Quinolones were the most frequently detected antibiotic group, present in 57.1-100% of biological samples, with concentrations ranging from 286.6 to 1655.3 µg/kg. Among the quinolones, enrofloxacin had the highest bioaccumulation factor (BAF) that can be defined as the ratio between the concentration of the analyzed antibiotic in an organism exposed from all sources and the concentration of the same antibiotic in water, during steady state (27,28). Its pollution increases public concern about human health and the safety of aquatic ecosystems. Enrofloxacin in sediment is absorbed by lipid-rich fish. Therefore, enrofloxacin bioaccumulation in fish habitats is positively related to fish lipid content (29). Ciprofloxacin, which WHO classifies as a critically important antibiotic, was investigated in fresh surface water, groundwater, saltwater, treated municipal wastewater, untreated municipal sewage, treated hospital wastewater, and untreated hospital wastewater. Among the 260 articles reporting ciprofloxacin contamination in watersheds, 111 studies were from Europe, 105 from Asia, and 32 from North America. Of these, 5 studies were conducted in South America and 7 in Africa. To determine the "probability of exceeding the observed ecotoxicological effect concentration" such as Predicted No-Effect Concentration (PNEC) and "antibiotic resistance rate" (ABR) for antibiotics in wastewater "probabilistic hazard assessments" are conducted. The PNEC is the concentration of an antibiotic in any environment below which adverse effects will most likely not occur during long-term or short-term exposure. The risk of antibiotic resistance (PNEC-ABR) is assessed by comparing the PNEC with the EEC (or PEC) or with the minimum effective concentration for the development or maintenance of antibiotic resistance. Since, there is no universally accepted or regulatory algorithm for obtaining a reliable ABR-PNEC for bacteria, it is estimated by applying evaluation factors to bacterial minimum inhibitory concentrations (MICs) (12). Remarkably, across all geographic regions, 58% of municipal effluents exceeded the ABR-PNEC for ciprofloxacin. Concentrations reported in surface waters exceeded the ABR-PNEC by 25%. Concentrations, which exceeded the ABR-PNEC were found to be by 31% in Europe,

29% in North America, and 67% in Africa (30). The interaction of ciprofloxacin with activated sludge from sewage treatment plants influences its migration and the control of associated risks. Sewage sludge removes over 96% of the ciprofloxacin from water through absorption, thereby increasing the ciprofloxacin concentration in the sediment. Extracellular proteins interact with ciprofloxacin. Ciprofloxacin contamination results in a decrease in soluble protein level, a significant elevation in extracellular protein concentration, and the formation of new extracellular proteins (31). The use of sewage sludge in ecological agriculture and the consumption of its products by humans pose a danger to human health by allowing humans to ingest products contaminated with antibiotics and increasing the resistance of commensal bacteria to these antibiotics. As an example, erythromycin has been detected in 1) wastewater, drinking water, groundwater, estuarine and coastal systems; 2) sewage sludge, biosolids, and sediments; and 3) tissues of aquatic organisms. In a study in China, 72.4% of surface waters were found to be contaminated; in a study in the United States, 85.4% were found to be contaminated; and in a study in Spain, 41.9% were found to be contaminated. The PNEC-ABR for erythromycin was found to be 33.3% higher in Asia, 20% in Europe, and 17.8% in North America (32). Antibiotic uptake by marine organisms, which are crucial for human nutrition, is specific to the compound type, the organism species, and the body tissues. Oxytetracycline accumulates in the organs, while oxolinic acid (a quinolone family antibiotic that inhibits DNA gyrase) accumulates in the gills (33). Indeed, among mollusks species collected from the Bohai Sea of China, antibiotic uptake appeared to vary depending on the species (34). High concentrations of fluoroquinolones have been found in liver and muscle tissue in fish from the marine aquaculture areas of the Pearl River Delta in South China (35). WTPs constitute a large reservoir of antibiotics, ARGs, and mobile genetic elements (MGEs). Mechanical and biological wastewater treatment methods are insufficient for removing these micro-pollutants. Exposure of ARB to heavy metals in the soil exacerbates adverse environmental impacts and accelerates the spread of resistance mechanisms. Excessive heavy metals in the environment and their high pressure on microorganisms directly affect the biodiversity of microbial communities living in the ecosystem (36). HGT is a significant ecological risk factor, leading to the co-selection of ARGs and metal resistance genes (MRGs) due to their co-localization on the same MGEs. Resistance arising from genetic changes triggered by environmental heavy metals in bacteria entering the environment through sewage systems and WTPs is an ecological factor that significantly reduces the effectiveness of antibiotics against bacterial infections (37). Significant correlation between abundance of ARGs and MGEs in a total of 164 ARGs from 50 soil samples, revealed the importance of HGT for ARG dissemination (38). HGT and co-localization of the host's MGE and MRG resistomes are shared between animal and human fecal samples. Many ARGs are shared by animals and human feces, in addition to the MGEs-containing class 1 integron, which are identified on animal feces, are carried by human pathogens (39).

Ecosystem and Antimicrobial Resistance

The rapid development of ARB is a global health security emergency. Current attention is centered on the mechanisms of transmission of ARB across species and the identification of environmental

reservoirs of antibiotic resistance in wastewater (40). To date, estimates disclose that 700,000 deaths occur each year due to the antimicrobial resistance; afterward, by 2050, 10 million deaths are expected every year (41). The genes selected are widely detected in wastewater samples, indicate various resistance mechanisms, and are as well located in different genetic elements, specifically in MGEs that substantially affect the spread of ARGs (42).

Resistance to antibiotics is acquired by bacteria via two routes: either vertical evolution (endogenous) or horizontal evolution (exogenous). Vertical evolution requires a spontaneous mutation in the bacterial genome that confers increased resistance to an antibiotic. Horizontal evolution occurs through the transfer of a resistance gene from a resistant bacterium to a susceptible one. The mechanisms of this route are conjugation, transduction, and transformation. During conjugation, the resistance plasmids containing ARGs are transferred between bacteria. Transformation is a kind of modification of the bacterial genome by the uptake and incorporation of exogenous DNA, while the transduction mechanism is the transfer of bacterial DNA via a viral vector (43). Wildlife contributes to the spread of antibiotic resistance across a variety of hosts and ecosystems at two levels. First, this transmission occurs locally, where animals living alongside humans can serve as reservoirs and vectors for the spread of antibiotic resistance. Second, migratory animals such as seagulls, fish and turtles contribute to the spread of antibiotic resistance across geographic regions and even between continents, posing a global health concern (4). Furthermore, the ARGs can be transferred between distantly related bacterial species and to the human pathogens and the bacteria that are capable of colonizing the human body (44). Ecological factors are regarded as potential hotspots for the spread of antimicrobial resistance. Antimicrobial contamination not only increases the resistance among environmental bacteria, but also directly impacts the virulence of antimicrobial-susceptible bacterial infections in patients by enhancing MICs of antibiotics and bacterial transmission (45). The proliferation of ARB results in alterations in the antibiotic susceptibility of the overall microbial population when antimicrobial levels are below the MIC. Consequently, resistant bacteria generally survive even in the availability of an antimicrobial level, which is sufficient to inhibit or kill microorganisms of the same species (46). Total enterococci and vancomycin-resistant enterococci are detected in 71% (34/48) and 4% (2/48) of reclaimed water samples, respectively, while *Enterococcus faecalis* (*E. faecalis*) is the major species. These enterococci in the reclaimed water present high antibiotic resistance at the point of water use as determined by using their MICs (47). When tomatoes were exposed to sulfamethoxazole and trimethoprim, they accumulated the antibiotics at 5.26 µg/kg and 3.40 µg/kg, respectively. Since the accumulated antibiotics, ARGs, and antibiotic-resistant pathogens (ARPs) are ingested via food, treatment of ARPs due to antibiotic resistance poses a significant challenge (48). Enrofloxacin, ciprofloxacin, ofloxacin, oxytetracycline, and erythromycin pose a high risk of selecting for antimicrobial resistance in marine culture water and/or sediment. Sulfonamide resistance genes *sul1* and *sul2* are the most common ones in water and sediment, whereas tetracycline resistance genes are widespread in the gills and intestines of fish. Species of *Proteobacteria*, *Actinomyces*, and *Cyanobacteria* are positively correlated with ARGs, suggesting that they may be potential hosts for

ARGs. Antibiotic resistance and human infection-related functions of the microbiome are significantly higher in fish than in marine culture (49). The development of antibiotic resistance in marine bacteria has been associated with antibiotic concentrations in wastewater discharges and aquaculture. Some recently identified genetic elements and resistance determinants for quinolones, tetracyclines, and β -lactamases have been found to be shared among aquatic bacteria, fish pathogens, and human pathogens. To demonstrate the ecological effects, 35 isolated bacterial strains were tested for resistance to the eight most frequently detected antibiotics. Resistance to the tested antibiotics was detected in 97.1% of the bacterial strains, of which 70.6% exhibited high-level resistance to the same antibiotics, particularly sulfonamides. Consequently, high bacterial resistance to antibiotics was significantly correlated with average antibiotic concentrations (50,51). ARB strains have been reported to be more prevalent in marine wildlife populations exposed to sewage contamination. Sharks sampled in the Florida Keys exhibited resistance to a broad range of antibiotics. Resistance to at least one antibiotic was detected in all fish species sampled from each of the six study sites. Multidrug resistance was also reported in most of the evaluated sites. Interspecific comparisons among redfish, *Sciaenops ocellatus*, and sharks from Louisiana offshore waters revealed a significantly higher prevalence of resistance depending on the age of the population (52). Evidence has emerged suggesting that ARB found in seabirds are of human origin. Several extended-spectrum β -lactamase-producing *Escherichia coli* (*E. coli*) isolated from birds clustered with human-derived isolates. Therefore, wild birds acquire *E. coli* with human-derived and human-resistant traits and thus, serve as an environmental reservoir with the potential to reinfect human populations (53).

The presence of ARGs in marine ecosystems indicates ecological changes caused by antibiotics. The presence of ARB phenotypes was investigated by direct antibiotic susceptibility testing in surface waters collected from 12 sources. According to the antibiotic susceptibility test results, resistance ranged from 17% to 100%. The antibiotic resistance profile against a total of 19 antibiotics was as follows: Amikacin (17%), gentamicin (17%), ciprofloxacin (33%), colistin (42%), linezolid (42%), tobramycin (42%), vancomycin (42%), ertapenem (67%), erythromycin (42%), meropenem (75%), rifampicin (75%), teicoplanin (75%), tetracycline (75%), trimethoprim (75%), fusidic acid (83%), aztreonam (92%), clindamycin (92%), penicillin (92%), and ceftiofur (100%). Multiple resistance, ranging from one to six antibiotic classes, was observed. It was found that antibacterial activity decreased sequentially starting from aminoglycosides to fluoroquinolones, glycopeptides, macrolides, tetracyclines and β -lactams (54). Although *E. coli* are inherently sensitive to practically all clinically significant antimicrobial agents, these bacteria have a considerable capacity to acquire resistance genes, usually through HGT. *E. coli* of animal origin often shows resistance to other, frequently older, antimicrobial agents, such as tetracyclines, phenicols, sulfonamides, trimethoprim, and fosfomycin. However, multi resistance plasmids and other MGEs, like transposons and gene cassettes in class 1 and class 2 integrons have significant effect in the dissemination of resistance genes among *E. coli* species (55). Evidence indicates that submarine discharges of treated effluents have negative impacts. The overall ARG content in the investigated microbiomes was dominated by genes encoding

multidrug efflux pumps, including MexAB-OprM, AcrEF-TolC, and MdtEF-TolC. The others are the modified peptidoglycan precursors, transporter genes conferring resistance to tetracycline, macrolide, and phenicol antibiotics, and the bla operon conferring β -lactam resistance. Several pathogenic genera introduced by effluents, like *Acinetobacter*, *Arcobacter*, *Bacteroides*, *Escherichia-Shigella*, *Klebsiella*, *Pseudomonas*, and *Salmonella*, show efflux pump-driven multidrug resistance, however *Acinetobacter*, *Salmonella*, *Bacteroides* and *Pseudomonas* emerge as predominant carriers of non-efflux ARGs conferring resistance to most of nine antibiotic classes (56). The core marine resistome is primarily involved in glycopeptide and multidrug resistance. Hosts for this resistome are largely restricted to the core marine microbiome, and gene transfer occurs more frequently within rather than between species, because of the presence of the phylogenetic barriers against the horizontal transfer of ARGs (57). Amongst multiple genetically polluted environments, water environments are the most likely ones constituting huge ARG reservoirs into which clinical and terrestrial bacteria flow and in which variety of human commensal bacteria develop (58).

Horizontal gene transfer

Bacteria have developed complicated strategies to counter pharmaceutical agents, such as decreasing antibiotic influx; reducing the drug's binding affinity or preventing drugs from effectively inhibiting the target enzyme; reducing intracellular concentration to sub-inhibitory levels via multidrug efflux pumps; inactivating the drug via hydrolysis of the antibiotic's functional group or transferring a chemical moiety to the antibiotic. These resistance mechanisms against antibiotics may be intrinsic to the species of bacteria or acquired from others via HGT (59). HGT, accomplished by conjugation by plasmids, transduction by bacteriophages, and natural transformation by extracellular DNA, allows genetic material to pass between strains and species to circumvent the normal vertical inheritance (60). In addition to these mechanisms, OMVs, which are abundant DNA reservoirs in aquatic environments, play an important role in the exchange of ARGs between bacteria (58,61). Of the three canonical mechanisms of HGT, conjugation is thought to have the greatest influence on the dissemination of ARGs (62). In fact, HGT is an epidemic infection model. The model is designed as a combination of quantitative measures of HGT and vertical gene transfer (VGT). It was determined that HGT is affected by the inhibitory mechanism of an antibiotic and its targets include donor, recipient alone or together, while VGT contributes vastly to the formation of transconjugants and consequently ARG spreading (63). In the environment, horizontal DNA transfer between multiple bacterial species and genera occurs by transformation and transduction, but primarily by conjugation. Conjugation is liable for the spread of genes coding for antibiotic resistance (64). Many environmental factors like nanomaterials, variety of oxidants, as well as light influence the horizontal transfer of ARGs in water environments (65). Furthermore, biofilm formation in water environments is considered to be hot spot for HGT of ARGs (58). HGT drives the evolution of untreatable "superbugs" by facilitating the accumulation of ARGs within the same cell. Thereby, HGT allows microbes to rapidly gain new capabilities and adapt to the environment. High rates of HGT have been detected among marine bacterial species. The frequency

of gene transfer detected in the oceans today is between a thousand- and a hundred-million-fold higher than previous estimates of HGT (60,66). ARGs are spread through HGT of ARG-associated MGEs (67). In addition, MGEs promote the assortment of AMR bacteria (68). Extracellular vesicles (EVs) are mediators of HGT and the spread of antibiotic resistance. In bacteria, the *mrdA/mrdB* pathway—a critical protein complex—consists of PBP2 (encoded by *mrdA*) and RodA (encoded by *mrdB*). Transcriptomic analysis has shown that *mrdA* and *mrdB* are key regulators of EV biogenesis. The *mrdA/mrdB* pathway has provided insights into the molecular mechanisms by which sub-inhibitory dose antibiotic exposure promotes the spread of antimicrobial resistance (69). New Delhi metallo- β -lactamase (NDM-1) is one of the most potent and widespread carbapenem-hydrolyzing enzymes; it is commonly found in bacteria and is secreted into the extracellular environment within outer membrane vesicles (OMVs). OMVs containing NDM-1 protect carbapenem-susceptible bacterial strains against meropenem. The secretion of NDM-1-containing OMVs ensures the survival of carbapenem-susceptible bacteria around the infection site (70). Indeed, OMVs from *E. coli* carrying NDM-1 not only display carbapenemase activity but also can protect the populations of carbapenem-susceptible bacteria (71). To reduce the spread of resistant strains and protect global public health, stronger antimicrobial governance and policy reforms are needed, including novel therapeutic approaches, such as phage therapy and nAbts. Effective interventions across multiple areas, from diagnosis to antibiotic administration policies, are critical to control the threat of antimicrobial resistance (72). On the other hand, studies support the possibility that ARGs in hospitals can spread through HGT among bacteria found in the microbiomes of animals such as cockroaches, fleas, or rats, thus these organisms

may be bioreactors for the horizontal transfer of ARGs among human pathogens (4). ARGs in plasmids can be transmitted across clinical and environmental boundaries. Network analysis revealed four β -lactam genes (*blaTEM-1*, *blaNDM-4*, *blaKPC-2*, and *blaSHV-1*) among 1029 plasmids and 45 clinical pathogens. This put forward that clinically alarming ARGs transfer expedite the propagation of antibiotic resistance in clinical pathogens (73). Packaged gene sets transferred via HGT allow bacteria to exchange multiple ARGs in a single transfer event (74). Horizontal transfer of CTX-M β -lactamase plasmid-borne *blaCTX-M* genes detected in *Klebsiella pneumoniae* (*K. pneumoniae*) isolates from hospital environment contributes to the spread of *K. pneumoniae* and creates an epidemiological hazard (75). In summary, HGT plays a significant role in the microbial ecology of antimicrobial resistance, as it allows the exchange of ARGs among taxonomically similar bacteria, and HGT rate depends on environmental conditions. In this context, multidrug-resistant soil bacteria have perfect nucleotide identity of genes from various human pathogens (76).

Clinical Perspective

During therapeutic antibiotic use, prolonged exposure of bacterial pathogens to high concentrations of antibiotics exerts significant selective pressure, leading to the development of more resistant bacteria. Although new antibiotics have shown a significant reduction in the persistence of bacterial infections, the development of resistance and biofilm formation generally minimize antibiotic effectiveness (77). The main disadvantages of conventional antimicrobial agents are their contribution to the development of multidrug resistance and their adverse effects. The biggest problem, however, is that newly synthesized antimicrobials do not have the

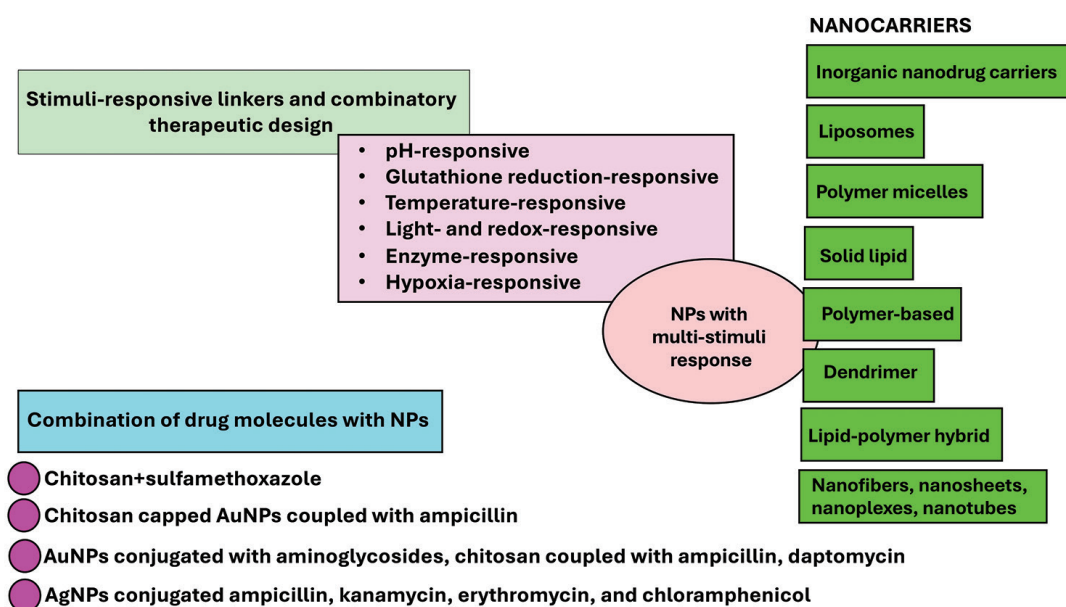


Figure 2. Types of nanoantibiotics (nAbts) that are currently used for drug delivery. The most significant advantage of nAbts, formulated to deliver drugs to infection sites using antibiotic-loaded nanocarriers, over traditional drug delivery systems is that nanoparticles (NPs) prevent the aggressive proliferation of resistant bacteria at the infection site by giving antibiotics novel functions through stimulus-triggered mechanisms.

Ag: Silver, Au: Gold, Sr: Stimuli responsive, nAbts: Nanoantibiotics, NP: Nanoparticle.

potential to provide a long-term solution (78). Combining antibiotics with NP-based carriers that offer better biofilm penetration, lower toxicity, and greater efficacy in combating bacterial resistance has become essential in overcoming antibiotic resistance, a global problem today. However, the clinical application of metal NP-based strategies against bacterial infections has been delayed (79) (Figure 2). In this context, the U.S. Food and Drug Administration (FDA)-approved- nAbts gold (Au) nanoclusters (NC), auranofin analogues are found to be effective against *Clostridium difficile* (*C. difficile*) infections, vancomycin-resistant *Enterococci*, methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) (auranofin has the capacity to eradicate the intracellular MRSA that is found inside infected macrophages), *Helicobacter pylori* (*H. pylori*) infections (synergistic with the amoxicillin via targeting both the reductive enzyme thioredoxin reductase and cell wall synthesis). Their anti-plasmodial activities likely result from direct inhibition of *Plasmodium falciparum* (*P. falciparum*) thioredoxin reductase. In addition, biofilm-associated bacteria can be treated with ultrasmall AuNPs (80). The daptomycin (Dap)-Au NCs hybrid structure demonstrates a stronger bactericidal effect toward MRSA which is a representative of multidrug-resistant bacteria, over the physically mixed Au NCs and daptomycin (Dap+Au NCs) (81).

It has been shown that the co-delivery of β -lactam antibiotics and β -lactamase inhibitors as nAbts, based on metal-carbenicillin framework-coated mesoporous silica NPs (MSN), is an effective method against MRSA. The constructed β -lactamase inhibitor-loaded nAbts are stable under physiological conditions. These nano formulations are capable of simultaneously releasing antibiotic molecules and the inhibitors at the site of bacterial infection. Thus, they achieve more effective elimination of ARB strains and biofilms. Ultimately, β -lactamase inhibitor-loaded nAbts exhibit greater penetration into biofilms, making them more effective at inhibiting MRSA (82). Folic acid tagged chitosan NPs are designed to function as "Trojan horses" to deliver vancomycin into bacterial cells. Since these NPs are biocompatible, biodegradable, semisynthetic polymers, they serve as valuable tools. Nanosized chitosan derivative loaded with vancomycin improves the transport of vancomycin across epithelial surfaces, thereby display antibacterial activity on vancomycin resistant *S. aureus* (83). Chitosan-capped AuNP coupled with ampicillin nAbt exhibited a two-fold increase in antimicrobial effectiveness compared with free ampicillin, while the antibiotic dosage was reduced to 50% of conventional usage (84). Ampicillin-functionalized AuNPs utterly demolish ampicillin-resistant bacteria (85). Similarly, the MIC of vancomycin-AuNPs against *S. aureus* is lower than that of free-form vancomycin. Therefore, the growth of intracellular *S. aureus* is successfully inhibited in vancomycin-AuNPs-engulfed macrophages (86). The combined antimicrobial effects of composite NPs and conventional antibiotics are more effective against bacteria resistant to conventional antibiotics. Although two different NPs are effective against the multidrug-resistant pathogens, each is difficult to functionalize with biomolecules and drugs. However, doxycycline-conjugated bimetallic NPs demonstrate substantially higher bactericidal activity against *Pseudomonas aeruginosa* (*P. aeruginosa*), *E. coli*, and *S. aureus*. This finding reveal that synergy of antibiotic with bimetallic NPs present a greatly efficient application (87). Compared to the ciprofloxacin, functionalized single-walled carbon nanotubes-ciprofloxacin show

substantial increment in the antibacterial activity by 16-fold for *S. aureus* and *P. aeruginosa* and by 8-fold for *E. coli* (88). In the majority of bacterial infections, virulence factors account for the rapid proliferation of microorganisms within the host. Therefore, novel therapeutic nAbts are designed with diverse linkers to confer novel functionalities, such as pH-sensitivity, temperature-sensitivity, light-sensitivity, redox-sensitivity, and enzyme-sensitivity. Stimulatory mechanisms launched by NPs increase the selectivity of nAbts activation and the inhibition of bacteria within the biofilm (89,90) (Figure 2).

Considering that the antimicrobial mechanism of conventional antibiotics is generally simple, bacterial resistance emerges rapidly. Contrarily, majority of the traditional antibiotic resistance mechanisms are void for metal NP (mNP)-based strategies (91). Based on existing data, nAbts provide a novel type of attack against which the bacteria currently lack a defense mechanism. Despite the resembling antibacterial efficiency, nAbts display lower toxicity in comparison to free-drug administration (92). Additionally, the protein corona formation significantly influences the uptake, biotransformation and biocompatibility as well as the cytotoxicity of NPs (93-95) (Figure 3). Opsonization of therapeutic NPs by protein adsorption results in substantial removal by reticuloendothelial cells, decreasing the bioavailability of circulating NPs at the targeted application site. Significantly reducing the immunotoxicity of NPs makes the NP carriers beneficial for antibiotic delivery (96,97).

In fact, antibiotic susceptibility characteristics define the relationship between a microorganism's *in vitro* susceptibility to the drug and the *in vivo* exposure of the microorganism to the drug (98). Metal oxide NPs used in conjunction with antibiotics not only reduce the toxicity of antibiotics but also improve their antibacterial efficacy (99). Antibiotic-loaded nanocarriers are designed to deliver drugs to infection sites, and mNP carriers protect antibiotics against resistance mechanisms developed by target bacteria (100) (Figure 3). Within mNP carriers, drugs are kept safe from harmful chemical reactions until they arrive their target *in vivo*, thus those carriers preserve the efficacy of the drugs and from antibiotic resistance mechanisms (101).

NP-based antibacterial drug delivery systems deliver the drug directly to the target site, thereby side effects are reduced. By proper NP carrier-mediated drug release, blood levels of the antibiotic at the targeted infection site can be sustained at the required effective concentrations for a prolonged period. This leads to improved stability and reduced frequency of antibiotic administration. Compared with the free drug at the same concentration, the drug delivered via an NP carrier has been shown to have a much greater inhibitory effect on cellular growth and is accompanied by prolonged drug release (102) (Figure 4). nAbts are administered alone or in combination with antibiotics to increase the likelihood of biofilm eradication or shorten the duration of treatment. Since polysaccharides and extracellular DNA (eDNA) associated proteins in the extracellular polymeric substance (EPS) matrix participate in the biofilm structural integrity, deoxyribonucleases (DNases) disrupt biofilms (103). EPS matrix-degrading enzymes facilitate dispersion of bacteria within biofilms, leading to more effective bacteriolysis when combined with antimicrobial agents. Dispersin, glucanohydrolases, glycoside hydrolases, and DNases enhance delivery of antimicrobial

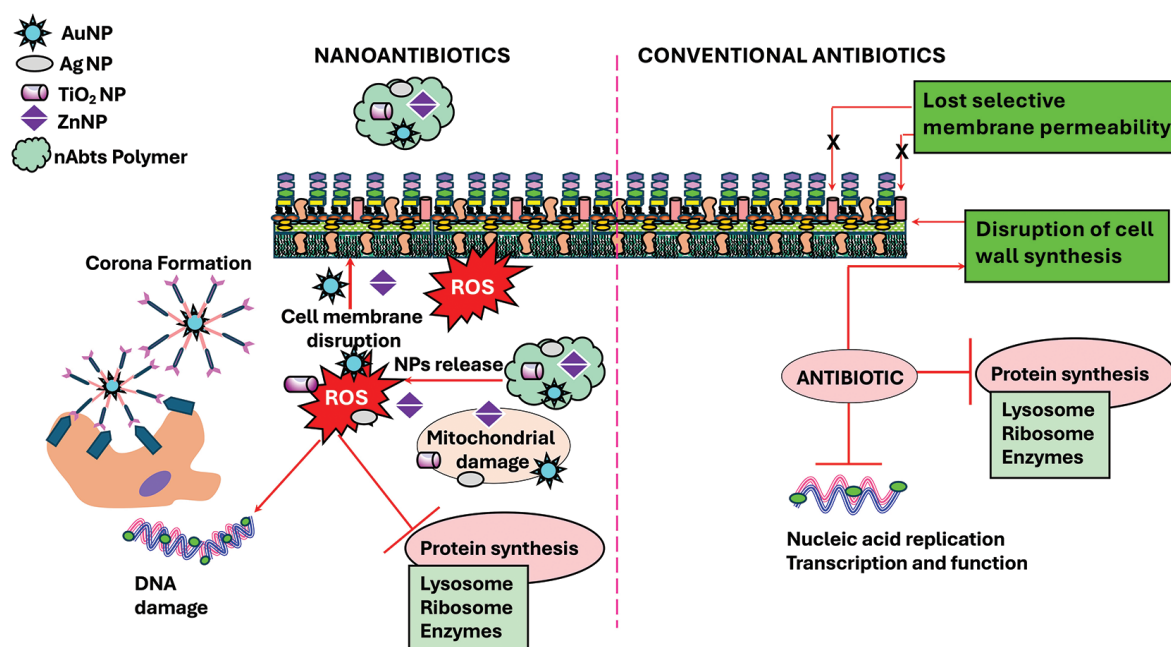


Figure 3. Conventional antibiotics disturb the bacterial membrane permeability and synthesis, as well as inhibit nucleic acid transcription and function. In contrast, nanoantibiotics (nABTs) act by impairing bacterial membranes via generating reactive oxygen species (ROS), leading to oxidative stress, disruption of transmembrane electron transport, and damage to mitochondria and DNA. The combination of antibiotics and metal oxide nanoparticles (mNPs) has been shown to reduce antibiotic toxicity while increasing their antibacterial efficacy.

Ag: Silver, Au: Gold, nABTs: Nanoantibiotics, NP: Nanoparticle, ROS: Reactive oxygen species, TiO₂: Titanium dioxide, Zn: Zinc.

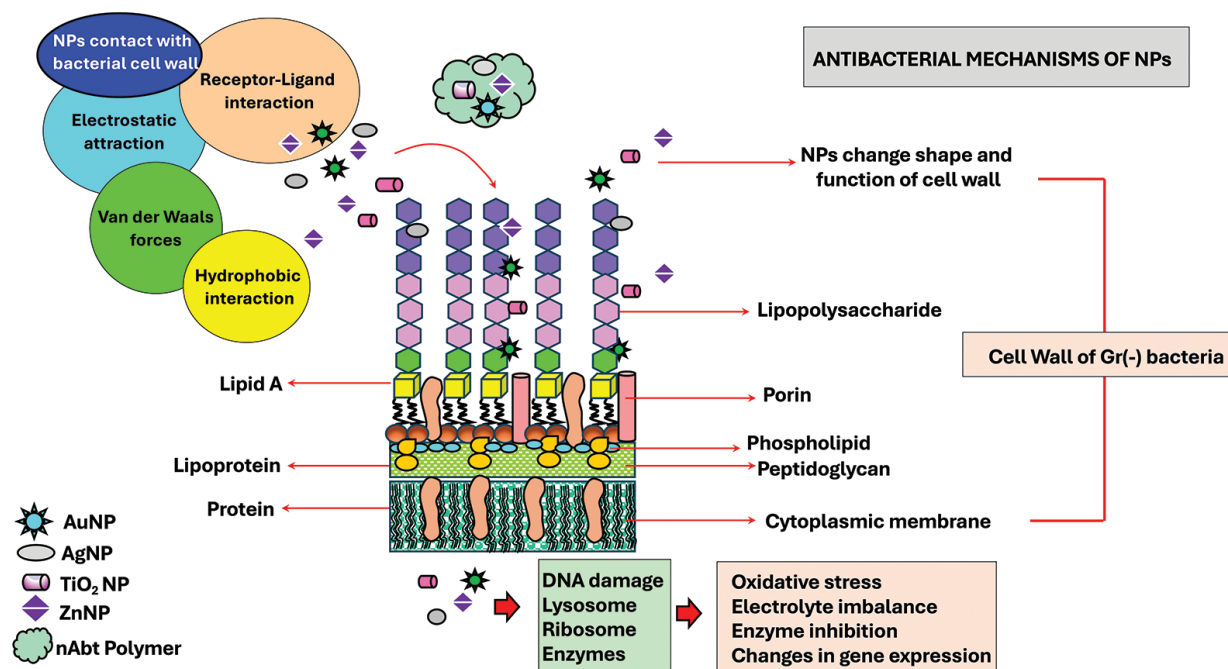


Figure 4. Nanoparticles (NPs) are required to be in contact with bacterial cells to achieve their antibacterial activity. Recognized modes of interaction between bacteria and nanoantibiotics (nABTs) include electrostatic attraction, van der Waals forces, receptor-ligand interactions, and hydrophobic interactions. Afterwards, NPs cross the bacterial membrane, assemble along metabolic pathways, and affect the structure and function of the cell membrane. Once inside the bacterial cell, NPs interact with essential components like DNA, lysosomes, ribosomes, and enzymes, causing oxidative stress, heterogeneous alterations, modified cell membrane permeability, electrolyte imbalances, enzyme inhibition, protein deactivation, as well as alteration in gene expression.

Ag: Silver, Au: Gold, Gr: Gram, TiO₂: Titanium dioxide, Zn: Zinc.

agents. Thereby they improve the killing potential of antibiotics or antimicrobial peptides, if they are administered in combination against pre-formed biofilms. Ciprofloxacin-loaded poly (lactic-co-glycolic acid) NPs, functionalized with DNase, exhibit antibiofilm activity against *P. aeruginosa* biofilms. Apart from releasing ciprofloxacin in a controlled manner, those NPs are capable of disassembling the biofilm by degrading the eDNA that stabilize the biofilm matrix (104). Drug delivery by lipid or polymer NPs is used to overcome biofilm resistance. The fusogenic liposomes maintain the release of antimicrobial agents in close proximity to the bacterial cells. These nanocarriers protect the antimicrobial compound from deleterious interactions with the matrix, enzymatic inactivation, and degradation by various bacterial and host components at the targeted infection site. Thereby, these nAbts readily penetrate the biofilm (105). Anti-biofilm peptides have broad-spectrum activity against both Gram-positive and Gram-negative bacteria within biofilms. Furthermore, they present vigorous synergy with conventional antibiotics (106). The polyethylene glycol shell, combined with its surface-adaptive charge, allows the mixed-shell polymeric micelles (MSPMs) to penetrate and accumulate extensively in *Staphylococcal* biofilms. After the binding of micellar nanocarriers to the *Staphylococcal* cell surface, bacterial enzymes degrade the MSPM core, and thus the nAbt releases its antimicrobial content resulting in the killing of bacteria in the deep layers of a biofilm. This mechanism creates an extremely effective pathway to control blood-accessible *Staphylococcal* biofilms with antimicrobials, through bypassing biofilm recalcitrance to antimicrobial penetration (107) (Figure 5). Through this novel technology, NP-based carriers can deliver antibiotics with improved biofilm penetration, thereby combating bacterial resistance.

CONCLUSION

Since current European legislation does not include limits on antimicrobial concentrations in treated wastewater from WTPs, various antimicrobials have been detected at concentrations above acceptable levels in numerous WTPs across many European countries. Therefore, agricultural workers and their families, product traders, agricultural product consumers, residents of areas and eco-system irrigated with wastewater are exposed to wastewater containing high levels of ARGs (108). Hence, antimicrobial resistance is a significant public health problem worldwide. Given the important and interdependent aspects of this resistance affecting humans, animals, and the environment, a “One Health” approach is necessary to combat it. The One Health concept encompasses environmental transmission pathways and ecological selection pressures that sustain resistance beyond host organisms. Whole genome sequencing performed to determine the ARG profile and phylogenetic relationship in strains isolated from humans and eco-system has revealed a significant overlap between major antibiotics and ARGs (109). This finding indicates that the pharmaceutical industry and healthcare systems will continue to struggle against ARB strains. Reducing the spread of antibiotic resistance and alleviating its burden in humans are critical. Combating antibiotic resistance and preventing the transmission of ARB and ARG to humans require implementing control strategies in four areas: (1) Diminishing the colonization capacity of exogenous ARB; (2) improving human colonization resistance and reducing HGT of ARG; (3) reversing ARB antibiotic resistance (110), and (4) determining the permissible antibiotic concentrations in wastewater discharged from wastewater plants and legally regulating wastewater treatment systems accordingly. Although new antibiotics have significantly reduced the

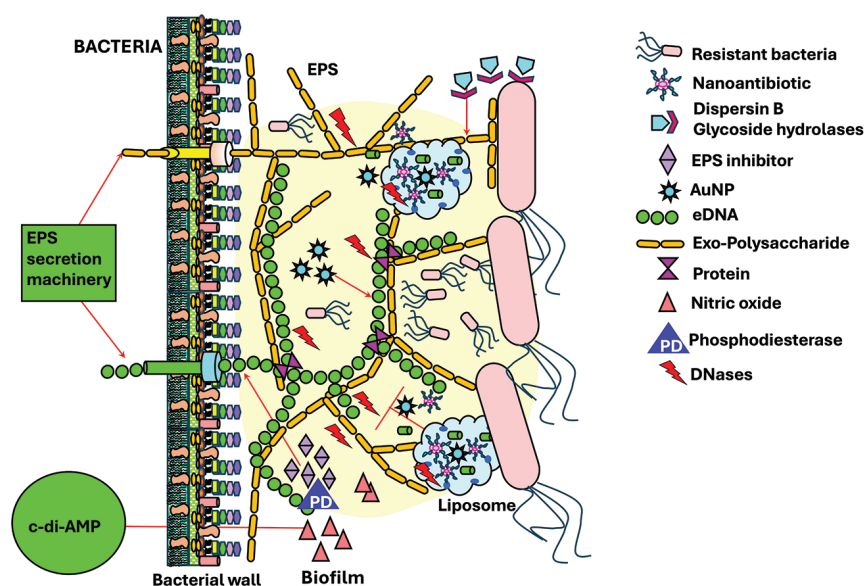


Figure 5. Bacterial biofilms, which have a structure rich in adhesine and extracellular matrix, protect microorganisms against conventional antibiotics. Nanoantibiotics (nAbts) target the extracellular polymeric substance (EPS) and decrease or completely remove the bacterial content of biofilm. Subsequent to the binding of micellar nanocarriers to bacterial cell surface, bacterial enzymes degrade mixed-shell-polymeric-micelles (MSPMs). Thus, nAbts penetrate and accumulate in bacterial biofilms. And then, antimicrobial compounds kill bacteria in the depth of a biofilm.

Au: Gold, EPS: Extracellular polymeric substance, c-di-AMP: Cyclic di-adenosine monophosphate, eDNA: Extracellular DNA, NP: Nanoparticle, PD: Phosphodiesterase.

persistence of bacterial infections, the development of resistance and biofilm formation generally reduce their effectiveness. Thus, newly synthesized antimicrobials lack the potential to provide a long-term solution. Combining antibiotics with NP-based carriers offers substantial advantages in overcoming antibiotic resistance, which is a global problem. Currently, only a small number of NP-based and hybrid nAbts have entered clinical practice. However, to address the problem of antibiotic resistance—which arises from the overuse of prophylactic or therapeutic antibiotics without appropriate medical indications, from the inappropriate selection of alternative antimicrobials, and from frequent switching between antimicrobial treatments—more clinical trials are needed to incorporate NP-based and hybrid nAbts into clinical practice.

Footnotes

Authorship Contributions

Concept: E.D.E., A.E., Design: E.D.E., A.E., Analysis or Interpretation: E.D.E., Literature Search: E.D.E., A.B.E., A.E., Writing: E.D.E., A.B.E., A.E.

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REFERENCES

- World Bank Group. Final report: drug-resistant infections a threat to our economic future [Internet]. Washington D.C.; 2017. Available from: <https://documents1.worldbank.org/curated/en/323311493396993758/pdf/final-report.pdf>
- Iskandar K, Molinier L, Hallit S, Sartelli M, Catena F, Coccolini F, et al. Drivers of antibiotic resistance transmission in low- and middle-income countries from a “one health” perspective—a review. *Antibiotics (Basel)*. 2020; 9: 372.
- World Bank Group. Operational framework for strengthening human, animal, and environmental public health systems at their interface [Internet]. Washington D.C.; 2018. Available from: <https://documents1.worldbank.org/curated/en/703711517234402168/pdf/123023-REVISED-PUBLIC-World-Bank-One-Health-Framework-2018.pdf>
- Laborda P, Sanz-García F, Ochoa-Sánchez LE, Gil-Gil T, Hernando-Amado S, Martínez JL. Wildlife and antibiotic resistance. *Front Cell Infect Microbiol*. 2022; 12: 873989.
- Lester Y, Avisar D, Gozlan I, Mamane H. Removal of pharmaceuticals using combination of UV/H₂O₂/O₃ advanced oxidation process. *Water Sci Technol*. 2011; 64: 2230-8.
- Jureczko M, Przysaś W. Ecotoxicity risk of presence of two cytostatic drugs: Bleomycin and vincristine and their binary mixture in aquatic environment. *Ecotoxicol Environ Saf*. 2019; 172: 210-5.
- Białk-Bielińska A, Mulkiewicz E, Stokowski M, Stolte S, Stepnowski P. Acute aquatic toxicity assessment of six anti-cancer drugs and one metabolite using biotest battery - biological effects and stability under test conditions. *Chemosphere*. 2017; 189: 689-98.
- Leksulchai V. Quantitation of anticancer drugs - cyclophosphamide and ifosfamide in urine and water sewage samples by gas chromatography-mass spectrometry. *Int J Occup Med Environ Health*. 2016; 29: 815-22.
- Grzesiuk M, Mielecki D, Pilżys T, Garbicz D, Marcinkowski M, Grzesiuk E. How cyclophosphamide at environmentally relevant concentration influences *Daphnia magna* life history and its proteome. *PLoS One*. 2018; 13: e0195366.
- Lutterbeck CA, Kern DI, Machado ÊL, Kümmerer K. Evaluation of the toxic effects of four anti-cancer drugs in plant bioassays and its potency for screening in the context of waste water reuse for irrigation. *Chemosphere*. 2015; 135: 403-10.
- WHO. Global research agenda for antimicrobial resistance in human health. Switzerland: antimicrobial resistance division World Health Organization; 2004.
- Straub JO, Oldenkamp R, Pfister T, Häner A. Environmental risk assessment for the active pharmaceutical ingredient mycophenolic acid in European surface waters. *Environ Toxicol Chem*. 2019; 38: 2259-78.
- Ashfaq M, Nawaz Khan K, Saif Ur Rehman M, Mustafa G, Faizan Nazar M, Sun Q, et al. Ecological risk assessment of pharmaceuticals in the receiving environment of pharmaceutical wastewater in Pakistan. *Ecotoxicol Environ Saf*. 2017; 136: 31-9.
- Langford K, Thomas KV. Input of selected human pharmaceutical metabolites into the Norwegian aquatic environment. *J Environ Monit*. 2011; 13: 416-21.
- Verlicchi P, Zambello E. Pharmaceuticals and personal care products in untreated and treated sewage sludge: occurrence and environmental risk in the case of application on soil - a critical review. *Sci Total Environ*. 2015; 538: 750-67.
- Martínez Bueno MJ, Boillot C, Munaron D, Fenet H, Casellas C, Gómez E. Occurrence of venlafaxine residues and its metabolites in marine mussels at trace levels: development of analytical method and a monitoring program. *Anal Bioanal Chem*. 2014; 406: 601-10.
- Klosterhaus SL, Grace R, Hamilton MC, Yee D. Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. *Environ Int*. 2013; 54: 92-9.
- Gonzalez-Rey M, Bebianno MJ. Effects of non-steroidal anti-inflammatory drug (NSAID) diclofenac exposure in mussel *Mytilus galloprovincialis*. *Aquat Toxicol*. 2014; 148: 221-30.
- Gonzalez-Rey M, Mattos JJ, Piazza CE, Bainy ACD, Bebianno MJ. Effects of active pharmaceutical ingredients mixtures in mussel *Mytilus galloprovincialis*. *Aquat Toxicol*. 2014; 153: 12-26.
- Solé M, Shaw JP, Frickers PE, Readman JW, Hutchinson TH. Effects on feeding rate and biomarker responses of marine mussels experimentally exposed to propranolol and acetaminophen. *Anal Bioanal Chem*. 2010; 396: 649-56.
- Fahmi A, Palin V, Zhong X, Yang YT, Watts S, Ashcroft DM, et al. Evaluation of the impact of COVID-19 pandemic on hospital admission related to common infections: risk prediction models to tackle antimicrobial resistance in primary care. *PLoS One*. 2024; 19: e0311515.
- WHO. 10 global health issues to track in 2021 [Internet]. 2020 [cited 2025 Aug 20]. Available from: <https://www.who.int/news-room/spotlight/10-global-health-issues-to-track-in-2021>
- Subramanya SH, Czyż DM, Acharya KP, Humphreys H. The potential impact of the COVID-19 pandemic on antimicrobial resistance and antibiotic stewardship. *Virusdisease*. 2021; 32: 330-7.
- Schlüter A, Szczepanowski R, Pühler A, Top EM. Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. *FEMS Microbiol Rev*. 2007; 31: 449-77.
- Liang X, Chen B, Nie X, Shi Z, Huang X, Li X. The distribution and partitioning of common antibiotics in water and sediment of the Pearl River Estuary, South China. *Chemosphere*. 2013; 92: 1410-6.
- Li S, Shi W, Li H, Xu N, Zhang R, Chen X, et al. Antibiotics in water and sediments of rivers and coastal area of Zhuhai City, Pearl River estuary, south China. *Sci Total Environ*. 2018; 636: 1009-19.

27. Bai Y, Meng W, Xu J, Zhang Y, Guo C. Occurrence, distribution and bioaccumulation of antibiotics in the Liao River Basin in China. *Environ Sci Process Impacts*. 2014; 16: 586-93.
28. Butnariu M. Chapter 2 - Heavy metals as pollutants in the aquatic Black Sea ecosystem. In: Dar GH, Bhat RA, Qadri H, Al-Ghamdy KM, Hakeem KR, editors. *Bacterial fish diseases* [Internet]. Academic Press; 2022 [cited 2026 Jun 28]. p. 31-57. Available from: <https://www.sciencedirect.com/science/article/pii/B9780323856249000038> doi:10.1016/B978-0-323-85624-9.00003-8
29. Xi F. The enrofloxacin pollution control from fish to environment. *Mar Pollut Bull*. 2024; 199: 115923.
30. Kelly KR, Brooks BW. Global aquatic hazard assessment of ciprofloxacin: exceedances of antibiotic resistance development and ecotoxicological thresholds. *Prog Mol Biol Transl Sci*. 2018; 159: 59-77.
31. Wang K, Gao D, Xu J, Cai L, Cheng J, Yu Z, et al. Interaction of ciprofloxacin with the activated sludge of the sewage treatment plant. *Environ Sci Pollut Res Int*. 2018; 25: 35064-73.
32. Schafhauser BH, Kristofco LA, de Oliveira CMR, Brooks BW. Global review and analysis of erythromycin in the environment: occurrence, bioaccumulation and antibiotic resistance hazards. *Environ Pollut*. 2018; 238: 440-51.
33. Le Bris H, Pouliquen H. Experimental study on the bioaccumulation of oxytetracycline and oxolinic acid by the blue mussel (*Mytilus edulis*). An evaluation of its ability to bio-monitor antibiotics in the marine environment. *Mar Pollut Bull*. 2004; 48: 434-40.
34. Li W, Shi Y, Gao L, Liu J, Cai Y. Investigation of antibiotics in mollusks from coastal waters in the Bohai Sea of China. *Environ Pollut*. 2012; 162: 56-62.
35. He X, Wang Z, Nie X, Yang Y, Pan D, Leung AOW, et al. Residues of fluoroquinolones in marine aquaculture environment of the Pearl River Delta, South China. *Environ Geochem Health*. 2012; 34: 323-35.
36. Epelde L, Lanzén A, Blanco F, Urich T, Garbisu C. Adaptation of soil microbial community structure and function to chronic metal contamination at an abandoned Pb-Zn mine. *FEMS Microbiol Ecol*. 2015; 91: 1-11.
37. Engin AB, Engin ED, Engin A. Effects of co-selection of antibiotic-resistance and metal-resistance genes on antibiotic-resistance potency of environmental bacteria and related ecological risk factors. *Environ Toxicol Pharmacol*. 2023; 98: 104081.
38. Zhao Y, Cocerva T, Cox S, Tardif S, Su JQ, Zhu YG, et al. Evidence for co-selection of antibiotic resistance genes and mobile genetic elements in metal polluted urban soils. *Sci Total Environ*. 2019; 656: 512-20.
39. Ma L, Xia Y, Li B, Yang Y, Li LG, Tiedje JM, et al. Metagenomic assembly reveals hosts of antibiotic resistance genes and the shared resistome in pig, chicken, and human feces. *Environ Sci Technol*. 2016; 50: 420-7.
40. Taylor NGH, Verner-Jeffreys DW, Baker-Austin C. Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? *Trends Ecol Evol*. 2011; 26: 278-84.
41. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations [Internet]. 2016 May. p. 1-81. Available from: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf
42. Wan X, Li Q, Li Z, Shi L, Pan Y, Li M, et al. MGE-associated ARGs exhibit higher expression efficiency than chromosomal non-MGE loci and predominantly contribute to resistance expression in pig farm wastewater. *Environ Int*. 2025; 202: 109653.
43. Laws M, Shaaban A, Rahman KM. Antibiotic resistance breakers: current approaches and future directions. *FEMS Microbiol Rev*. 2019; 43: 490-516.
44. Cycoń M, Mrozik A, Piotrowska-Seget Z. Antibiotics in the soil environment-degradation and their impact on microbial activity and diversity. *Front Microbiol*. 2019; 10: 338.
45. Le Page G, Gunnarsson L, Snape J, Tyler CR. Integrating human and environmental health in antibiotic risk assessment: a critical analysis of protection goals, species sensitivity and antimicrobial resistance. *Environ Int*. 2017; 109: 155-69.
46. Bengtsson-Palme J, Larsson DGJ. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. *Environ Int*. 2016; 86: 140-9.
47. Bouissou-Schurtz C, Houeto P, Guerbet M, Bachelot M, Casellas C, Mauclair AC, et al. Ecological risk assessment of the presence of pharmaceutical residues in a French national water survey. *Regul Toxicol Pharmacol*. 2014; 69: 296-303.
48. Christou A, Agüera A, Bayona JM, Cytryn E, Fotopoulos V, Lambropoulou D, et al. The potential implications of reclaimed wastewater reuse for irrigation on the agricultural environment: the knowns and unknowns of the fate of antibiotics and antibiotic resistant bacteria and resistance genes - a review. *Water Res*. 2017; 123: 448-67.
49. He LX, He LY, Gao FZ, Wu DL, Ye P, Cheng YX, et al. Antibiotics, antibiotic resistance genes and microbial community in grouper mariculture. *Sci Total Environ*. 2022; 808: 152042.
50. Cabello FC, Godfrey HP, Tomova A, Ivanova L, Dölz H, Millanao A, et al. Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environ Microbiol*. 2013; 15: 1917-42.
51. Zheng S, Qiu X, Chen B, Yu X, Liu Z, Zhong G, et al. Antibiotics pollution in Jiulong River estuary: source, distribution and bacterial resistance. *Chemosphere*. 2011; 84: 1677-85.
52. Blackburn JK, Mitchell MA, Blackburn MCH, Curtis A, Thompson BA. Evidence of antibiotic resistance in free-swimming, top-level marine predatory fishes. *J Zoo Wildl Med*. 2010; 41: 7-16.
53. Bonnedahl J, Drobni M, Gauthier-Clerc M, Hernandez J, Granholm S, Kayser Y, et al. Dissemination of *Escherichia coli* with CTX-M type ESBL between humans and yellow-legged gulls in the south of France. *PLoS One*. 2009; 4: e5958.
54. Moore JE, Rao JR, Moore PJA, Millar BC, Goldsmith CE, Loughrey A, et al. Determination of total antibiotic resistance in waterborne bacteria in rivers and streams in Northern Ireland: can antibiotic-resistant bacteria be an indicator of ecological change? *Aquat Ecol*. 2010; 44: 349-58.
55. Poirel L, Madec JY, Lupo A, Schink AK, Kieffer N, Nordmann P, et al. Antimicrobial resistance in *Escherichia coli*. *Microbiol Spectr*. 2018; 6.
56. Kvesić M, Kalinić H, Dželalija M, Šamanić I, Andričević R, Maravić A. Microbiome and antibiotic resistance profiling in submarine effluent-receiving coastal waters in Croatia. *Environ Pollut*. 2022; 292: 118282.
57. Xu N, Qiu D, Zhang Z, Wang Y, Chen B, Zhang Q, et al. A global atlas of marine antibiotic resistance genes and their expression. *Water Res*. 2023; 244: 120488.
58. Abe K, Nomura N, Suzuki S. Biofilms: hot spots of horizontal gene transfer (HGT) in aquatic environments, with a focus on a new HGT mechanism. *FEMS Microbiol Ecol*. 2020; 96: fiae031.
59. GBD 2021 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050. *Lancet*. 2024; 404: 1199-226.

60. Lermniaux NA, Cameron ADS. Horizontal transfer of antibiotic resistance genes in clinical environments. *Can J Microbiol.* 2019; 65: 34-44.
61. Biller SJ, Schubotz F, Roggensack SE, Thompson AW, Summons RE, Chisholm SW. Bacterial vesicles in marine ecosystems. *Science.* 2014; 343: 183-6.
62. von Wintersdorff CJH, Penders J, van Niekerk JM, Mills ND, Majumder S, van Alphen LB, et al. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front Microbiol.* 2016; 7: 173.
63. Li B, Qiu Y, Song Y, Lin H, Yin H. Dissecting horizontal and vertical gene transfer of antibiotic resistance plasmid in bacterial community using microfluidics. *Environ Int.* 2019; 131: 105007.
64. Wolska KI. Horizontal DNA transfer between bacteria in the environment. *Acta Microbiol Pol.* 2003; 52: 233-43.
65. Li W, Zhang G. Detection and various environmental factors of antibiotic resistance gene horizontal transfer. *Environ Res.* 2022; 212: 113267.
66. McDaniel LD, Young E, Delaney J, Ruhnu F, Ritchie KB, Paul JH. High frequency of horizontal gene transfer in the oceans. *Science.* 2010; 330: 50.
67. Rodríguez-Beltrán J, DelaFuente J, León-Sampedro R, MacLean RC, San Millán Á. Beyond horizontal gene transfer: the role of plasmids in bacterial evolution. *Nat Rev Microbiol.* 2021; 19: 347-59.
68. Shintani M, Vestergaard G, Milaković M, Kublik S, Smalla K, Schlöter M, et al. Integrins, transposons and IS elements promote diversification of multidrug resistance plasmids and adaptation of their hosts to antibiotic pollutants from pharmaceutical companies. *Environ Microbiol.* 2023; 25: 3035-51.
69. Li J, Zuo J, Xu H, Yang J, Hu Y, Han Y, et al. Sub-inhibitory gentamicin promotes extracellular vesicles biogenesis and bla_{NDM} dissemination in carbapenem-resistant *Escherichia coli* via mrdA/mrdB pathway. *Vet Microbiol.* 2025; 310: 110704.
70. Martínez MMB, Bonomo RA, Vila AJ, Maffía PC, González LJ. On the offensive: the role of outer membrane vesicles in the successful dissemination of New Delhi metallo- β -lactamase (NDM-1). *mBio.* 2021; 12: e0183621.
71. González LJ, Bahr G, Nakashige TG, Nolan EM, Bonomo RA, Vila AJ. Membrane anchoring stabilizes and favors secretion of New Delhi metallo- β -lactamase. *Nat Chem Biol.* 2016; 12: 516-22.
72. Aljohani MS, Harun-Ur-Rashid M, Selim S. Emerging threats: antimicrobial resistance in extended-spectrum beta-lactamase and carbapenem-resistant *Escherichia coli*. *Microb Pathog.* 2025; 200: 107275.
73. Wang X, Zhang H, Yu S, Li D, Gillings MR, Ren H, et al. Inter-plasmid transfer of antibiotic resistance genes accelerates antibiotic resistance in bacterial pathogens. *ISME J.* 2024; 18: wrad032.
74. Slizovskiy IB, Mukherjee K, Dean CJ, Boucher C, Noyes NR. Mobilization of antibiotic resistance: are current approaches for colocalizing resistomes and mobilomes useful? *Front Microbiol.* 2020; 11: 1376.
75. Cheng J, Wang Q, Chen Y, Ye Y, Li H, Li X, et al. Phenotypic and molecular characterization of a novel beta-lactamase carried by *Klebsiella pneumoniae*, CTX-M-72, derived from CTX-M-3. *J Gen Appl Microbiol.* 2009; 55: 207-16.
76. Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MOA, Dantas G. The shared antibiotic resistome of soil bacteria and human pathogens. *Science.* 2012; 337: 1107-11.
77. Fernandez-Moure JS, Evangelopoulos M, Colvill K, Van Eps JL, Tasciotti E. Nanoantibiotics: a new paradigm for the treatment of surgical infection. *Nanomedicine (Lond).* 2017; 12: 1319-34.
78. Edson JA, Kwon YJ. Design, challenge, and promise of stimuli-responsive nanoantibiotics. *Nano Converg.* 2016; 3: 26.
79. Engin AB, Engin A. Nanoantibiotics: a novel rational approach to antibiotic resistant infections. *Curr Drug Metab.* 2019; 20: 720-41.
80. Ndugire W, Raviranga NGH, Lao J, Ramström O, Yan M. Gold nanoclusters as nanoantibiotic auranofin analogues. *Adv Healthc Mater.* 2022; 11: e2101032.
81. Zheng Y, Liu W, Chen Y, Li C, Jiang H, Wang X. Conjugating gold nanoclusters and antimicrobial peptides: from aggregation-induced emission to antibacterial synergy. *J Colloid Interface Sci.* 2019; 546: 1-10.
82. Duan F, Feng X, Jin Y, Liu D, Yang X, Zhou G, et al. Metal-carbenicillin framework-based nanoantibiotics with enhanced penetration and highly efficient inhibition of MRSA. *Biomaterials.* 2017; 144: 155-65.
83. Chakraborty SP, Sahu SK, Mahapatra SK, Santra S, Bal M, Roy S, et al. Nanoconjugated vancomycin: new opportunities for the development of anti-VRSA agents. *Nanotechnology.* 2010; 21: 105103.
84. Chamundeeswari M, Sobhana SSL, Jacob JP, Kumar MG, Devi MP, Sastry TP, et al. Preparation, characterization and evaluation of a biopolymeric gold nanocomposite with antimicrobial activity. *Biotechnol Appl Biochem.* 2010; 55: 29-35.
85. Brown AN, Smith K, Samuels TA, Lu J, Obare SO, Scott ME. Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of *Pseudomonas aeruginosa* and *Enterobacter aerogenes* and methicillin-resistant *Staphylococcus aureus*. *Appl Environ Microbiol.* 2012; 78: 2768-74.
86. Lai HZ, Chen WY, Wu CY, Chen YC. Potent antibacterial nanoparticles for pathogenic bacteria. *ACS Appl Mater Interfaces.* 2015; 7: 2046-54.
87. Fakhri A, Tahami S, Naji M. Synthesis and characterization of core-shell bimetallic nanoparticles for synergistic antimicrobial effect studies in combination with doxycycline on burn specific pathogens. *J Photochem Photobiol B.* 2017; 169: 21-6.
88. Assali M, Zaid AN, Abdallah F, Almasri M, Khayyat R. Single-walled carbon nanotubes-ciprofloxacin nanoantibiotic: strategy to improve ciprofloxacin antibacterial activity. *Int J Nanomedicine.* 2017; 12: 6647-59.
89. Canaparo R, Foglietta F, Giuntini F, Pepa CD, Dosio F, Serpe L. Recent developments in antibacterial therapy: focus on stimuli-responsive drug-delivery systems and therapeutic nanoparticles. *Molecules.* 2019; 24: 1991.
90. de la Rica R, Aili D, Stevens MM. Enzyme-responsive nanoparticles for drug release and diagnostics. *Adv Drug Deliv Rev.* 2012; 64: 967-78.
91. Wang YL, He M, Miron RJ, Chen AY, Zhao YB, Zhang YF. Temperature/pH-sensitive nanoantibiotics and their sequential assembly for optimal collaborations between antibacterial and immunoregulation. *ACS Appl Mater Interfaces.* 2017; 9: 31589-99.
92. Wolfram J, Zhu M, Yang Y, Shen J, Gentile E, Paolino D, et al. Safety of nanoparticles in medicine. *Curr Drug Targets.* 2015; 16: 1671-81.
93. Neagu M, Piperigkou Z, Karamanou K, Engin AB, Docea AO, Constantin C, et al. Protein bio-corona: critical issue in immune nanotoxicology. *Arch Toxicol.* 2017; 91: 1031-48.
94. Cedervall T, Lynch I, Lindman S, Berggård T, Thulin E, Nilsson H, et al. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proc Natl Acad Sci USA.* 2007; 104: 2050-5.
95. Ge C, Du J, Zhao L, Wang L, Liu Y, Li D, et al. Binding of blood proteins to carbon nanotubes reduces cytotoxicity. *Proc Natl Acad Sci USA.* 2011; 108: 16968-73.

96. Dobrovolskaia MA, McNeil SE. Immunological properties of engineered nanomaterials. *Nat Nanotechnol.* 2007; 2: 469-78.
97. Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R. Biodegradable long-circulating polymeric nanospheres. *Science.* 1994; 263: 1600-3.
98. Mouton JW, Ambrose PG, Canton R, Drusano GL, Harbarth S, MacGowan A, et al. Conserving antibiotics for the future: new ways to use old and new drugs from a pharmacokinetic and pharmacodynamic perspective. *Drug Resist Updat.* 2011; 14: 107-17.
99. Muzammil S, Hayat S, Fakhar-E-Alam M, Aslam B, Siddique MH, Nisar MA, et al. Nanoantibiotics: Future nanotechnologies to combat antibiotic resistance. *Front Biosci (Elite Ed).* 2018; 10: 352-74.
100. Kalhapure RS, Suleman N, Mocktar C, Seedat N, Govender T. Nanoengineered drug delivery systems for enhancing antibiotic therapy. *J Pharm Sci.* 2015; 104: 872-905.
101. Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *J Control Release.* 2011; 156: 128-45.
102. Liu JL, Zhang WJ, Li XD, Yang N, Pan WS, Kong J, et al. Sustained-release genistein from nanostructured lipid carrier suppresses human lens epithelial cell growth. *Int J Ophthalmol.* 2016; 9: 643-9.
103. Okshevsky M, Regina VR, Meyer RL. Extracellular DNA as a target for biofilm control. *Curr Opin Biotechnol.* 2015; 33: 73-80.
104. Baelo A, Levato R, Julián E, Crespo A, Astola J, Gavalda J, et al. Disassembling bacterial extracellular matrix with DNase-coated nanoparticles to enhance antibiotic delivery in biofilm infections. *J Control Release.* 2015; 209: 150-8.
105. Forier K, Raemdonck K, De Smedt SC, Demeester J, Coenye T, Braeckmans K. Lipid and polymer nanoparticles for drug delivery to bacterial biofilms. *J Control Release.* 2014; 190: 607-23.
106. Pletzer D, Coleman SR, Hancock RE. Anti-biofilm peptides as a new weapon in antimicrobial warfare. *Curr Opin Microbiol.* 2016; 33: 35-40.
107. Liu Y, Busscher HJ, Zhao B, Li Y, Zhang Z, van der Mei HC, et al. Surface-adaptive, antimicrobially loaded, micellar nanocarriers with enhanced penetration and killing efficiency in staphylococcal biofilms. *ACS Nano.* 2016; 10: 4779-89.
108. Adegoke AA, Amoah ID, Stenström TA, Verbyla ME, Mihelcic JR. Epidemiological evidence and health risks associated with agricultural reuse of partially treated and untreated wastewater: a review. *Front Public Health.* 2018; 6: 337.
109. Li C, Lee LHA, Nanayakkara Sapugahawatte D, Lee WS, Cheung IYY, Chow VCY, et al. One health approach to characterize antimicrobial resistance in *Morganella morganii* in humans, freshwater fish and pigs. *JAC Antimicrob Resist.* 2026; 8: dlag064.
110. Ding D, Wang B, Zhang X, Zhang J, Zhang H, Liu X, et al. The spread of antibiotic resistance to humans and potential protection strategies. *Ecotoxicol Environ Saf.* 2023; 254: 114734.