Chapter

Antibiotic Resistance Breakers and Nano-Antibiotics in Mediating Antimicrobial Resistance

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Abstract

Antibiotic resistance is increasing at an alarming rate and is now widely recognized as a global issue that requires urgent attention. Globally, the demand for new drugs has increased due to multidrug-resistant pathogens and emerging viruses. One promising avenue of research involves antibiotic resistance breakers (ARBs), which may or may not have direct antibacterial effects and can either be co-administered with or conjugated with failing antibiotics. This strategy may increase an antibiotic's spectrum and its efficacy against bacteria that have acquired resistance against it and reduce the dosage necessary for an antibiotic. In this chapter, we have discussed antibiotic resistance breakers, their classification, and mechanisms of action in combating microbial resistance. Moreover, this chapter will also focus on the nanotechnological approach, a novel delivery platform using nano-carriers used to overcome the permeability barrier encountered in resistant bacteria. Nano-carriers are also used to selectively deliver high concentrations of antibiotics locally, thus avoiding systemic side effects. Several strategies have been studied to deliver antibiotics such as the use of antimicrobial polymers, nanoparticles, and liposomes. The current study will help to understand how the resistance ability of bacteria can be overcome or reversed through antibiotic resistance breakers and nano-antibiotics.

Keywords: antibiotic resistance, antibiotic resistance breakers, modifying enzyme inhibitors, membrane permeabilizers, efflux pump inhibitors, nano-antibiotics

1. Introduction

Human history has shown that microbes and the human population have been in a long-lasting fight around the globe from facing severe pandemic situations with the discovery of antibiotic substances. Antibiotics have been employed for several decades as the most effective treatment against deadly bacterial infections. An antibiotic is a substance that inhibits bacterial growth by one of several mechanisms including preventing the synthesis of the cell wall, inhibiting the formation of folic acid, interfering with the central dogma of the bacterial cells, and changing the permeability through destruction of the bacterial plasma membranes [1]. In order to

demonstrate effective antibacterial activity, the three requirements must be fulfilled. These include the bacterial cells should have an intact target site, the presence of active and non-metabolizing antibiotic drugs, and a satisfactory amount of drug should be present at the target site inside the bacterial cell. If any of these conditions are modified by a bacterial strain, then it might develop resistance in that strain to a specific antibacterial drug [2]. Conversely, inside the cell, antibacterial drugs are poorly utilized because they are unable to reach the target site in a sufficient amount of time. For this reason, larger doses of antibiotics are being employed and this may also cause the drugs to spread systemically to non-targeted tissues. This over-and/or misuse of antibiotics leads to the emergence of antibiotic resistance [3].

The ever-increasing antibacterial resistance due to the evolution of resistant bacterial phenotypes is continuously threatening the world health benefits obtained from antibiotic medications. According to Singh [4], World Health Organization (WHO) has stated it as the highlighted issue of the twenty first century that needs to be solved soon. Mechanisms of antimicrobial resistance undertaken by a bacterial strain may be acquired because of genetic mutation(s). Transposable elements in the bacterial genome encode several resistant genes making that phenotype resistant [5]. The defensive approaches that lead to antimicrobial resistance involve the restriction of an antibiotic to enter the cell by disruption of the plasma membrane, pumping out of the drug by overexpression of the efflux pump, alteration of the binding sites for the antibiotic, and modification of the enzyme targeted by the antibacterial drug [2].

Studies have shown that employing anti-bacterial substances in the agriculture sector resulted in the formation of resistant strains of bacteria leading to one of the main reasons for developing antimicrobial resistance in the human population [6]. Antibiotic resistance is treated by various approaches, one such approach that has been employed is the antimicrobial resistance breakers. These breakers are non-reactive and have little or no antibacterial activity. But when they are employed in association with other antibiotic, particularly with those against which resistance has been developed, these adjuvants result in potentiating their antibacterial activity [7].

To overcome the adverse effects caused by the misuse of antibiotic substances, studies are being conducted to enhance the *in vivo* antibacterial activity of drugs by utilizing novel technologies for effective drug delivery. Nanotechnology is one of the novel approaches used in various sectors of life. Its application in the medical and drug discovery field has paved new ways to treat several infections and it is also utilized in combating bacterial resistance. This chapter will provide a basic understanding of antimicrobial resistance breakers and highlight the approaches that can be used to overcome this challenging issue of the twenty first century. The main objective of this chapter is to explain how nanoparticles can help antibiotics to enhance the antibacterial effect at the nanoscale by understanding the molecular mechanism and functional characteristics of nano-antibiotics (nAbts). It is also expected that these understandings will pave more ways in the development of effective nano-antibiotics in the research of future applications, especially in the biomedical field.

2. Antibiotic resistance breakers

The ever-increasing bacterial strains make the phenomenon of antibiotic resistance a challenging issue around the globe. To counter this critical situation, novel treatment approaches are being exploited. One such is the use of antibiotic resistance breakers (ARBs). These are non-reactive compounds that are administered

along with an antibiotic to overcome the bacterial resistance to that antibiotic either by improving the antibacterial drug efficiency or by inhibiting the resistance mechanism [7]. These ARBs have the ability to combat bacterial resistance *via* several mechanisms like enhancing the uptake of antibiotics, obstructing the efflux of drug, signaling pathways, preventing the modification of both drug and target sites, and formation of biofilm [8, 9]. The effective ABRs should possess the following one or more characteristics: (a) they should have direct antibacterial action even though they are not employed in clinical settings as antibiotics, (b) they may improve antibiotic effectiveness and/or counteract drug resistance mechanisms, (c) they may aid in the clearance of the infection by interacting with host targets to trigger host defense mechanisms, such as encouraging autophagy or blocking pro-inflammatory toll-like receptors (TLRs) or encouraging autophagy [10]. The exploitation of antibiotic adjuvants is a cost-effective therapy to combat antibiotic resistance by conjugating with ineffective antibiotics. Dual antibiotic therapy, which has previously been successful due to the additive or synergistic effects of the individual antibiotic agents, is where the concept of conjugation of ARBs with conventional antibiotics emerged [11]. Successfully co-administered ARBs should improve the effects of antibiotics by enabling the use of lower antibiotic doses. The term minimum inhibitory concentration (MIC), which refers to the lowest concentration of a substance needed to prevent the pathogenic species from growing visibly under specific conditions, is helpful in this context. The more effective ARBs reduce the MICs of antibiotics more effectively than antibiotic monotherapy [7]. Due to this reason, researches are still carrying out great efforts to find more antibiotic adjuvants. Moreover, this seems to be a quite appealing approach, as the decreased antibiotic selection pressure may delay the onset of resistance and help to reduce the side effects that patients receive after antibiotic monotherapy. Approaches which have been employed to combat bacterial resistance are depicted in **Figure 1**.

2.1 Classification of antibiotic resistance breakers

The antibiotic resistance breaker can be classified generally into two main categories described below:

2.1.1 Class I ARBs

The ARBs grouped under this class are those that interfere with bacterial functioning by enhancing the antibacterial potential. Based on their mechanisms of action, they are further classified into two subgroups (i.e., IA and IB) [12]. Class IA compounds are those that directly target the antibacterial resistance pathway such as modification of the inhibitor enzymes, membrane permeabilization, and efflux pump inhibition [8]. They are the only adjuvants currently employed in clinical practice. The most effective Class IA adjuvants include beta-lactamases inhibitors and the enzymes involved in the hydrolytic inactivation of cephalosporins, carbapenem, and penicillin [12]. Whereas class IB adjuvants are those that, instead of directly inhibiting particular resistant elements, increase antibiotic action by evading intrinsic (e.g., metabolic pathways or physiology) or extrinsic resistance mechanisms. This class of ARBs is being developed by screening the bacterial cell in order to boost the antibacterial mechanisms and to target other parameters, which are not related to the target site. For instance, loperamide is employed with tetracycline to enhance the uptake of tetracycline inside the cell by raising the pH of the intracellular environment and

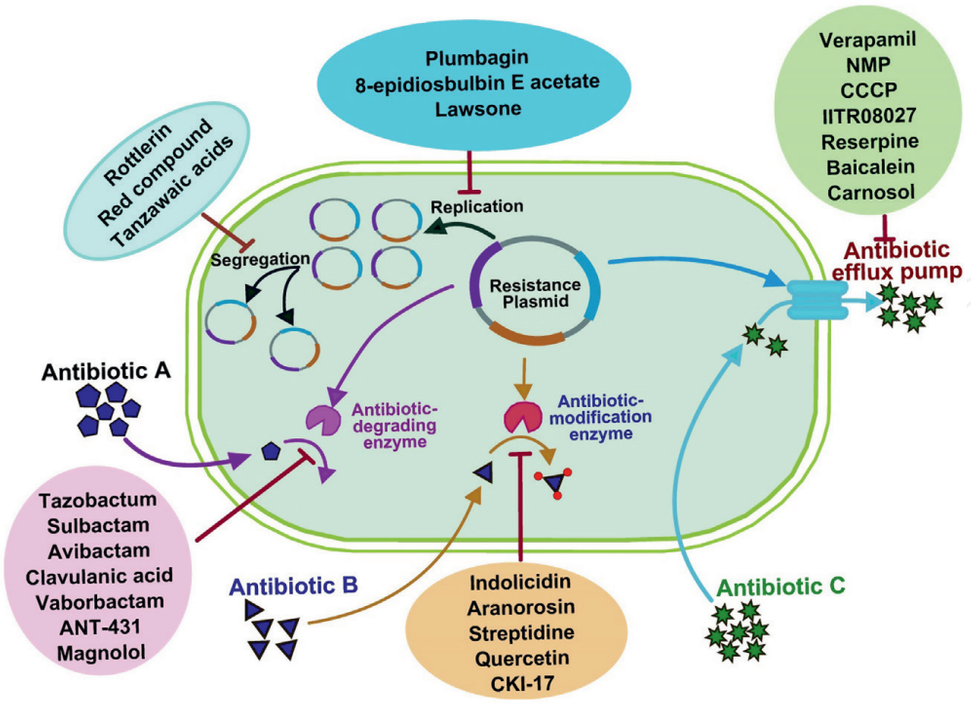

Figure 1.
Approaches to combat antimicrobial resistance [5].

reducing the electric component of the proton motive force. Hence, in this case, loperamide adjuvant works indirectly to boost the influx of antibiotics [8]. The adjuvants belonging to this group are presently being discovered in preclinical models [12]. The structures of some of the class I antibiotic resistance breakers are given in **Figure 2**.

2.1.2 Class II ARBs

This class contains those ARBs which enhance the efficiency of antibiotic drugs by changing the host biology via interfering with its immune system [13]. Small molecules that directly interfere with the host's innate immune system have seemed to be very effective in combating bacterial resistance [14]. For instance, streptazolin extracts cause the production of antimicrobial cytokines and are responsible for the activation of the synthesis of nuclear factor-κB, resulting in the phagocytosis of *Streptococcus pneumonia*. Presently, several loci which are responsible for resistance against infection in the host genome have been identified using the CRISPR-Cas9 genome editing technique [8]. These loci have served as a model for the exploration of class II antibiotic resistance breakers, imitating the influence of factors causing host resistance and providing the pathway to discover novel ways to combat antibiotic resistance [15].

2.2 Mechanism of antibiotic resistance breakers

2.2.1 Modification of enzyme inhibitors

Enzymes involved in molecular mechanisms are chiefly responsible for causing antimicrobial resistance by modifying the antibiotic [5]. These modifying enzymes are broadly classified into two groups depending upon the two factors, i.e., the antibiotic that acts as a substrate and the metabolic pathway on which these

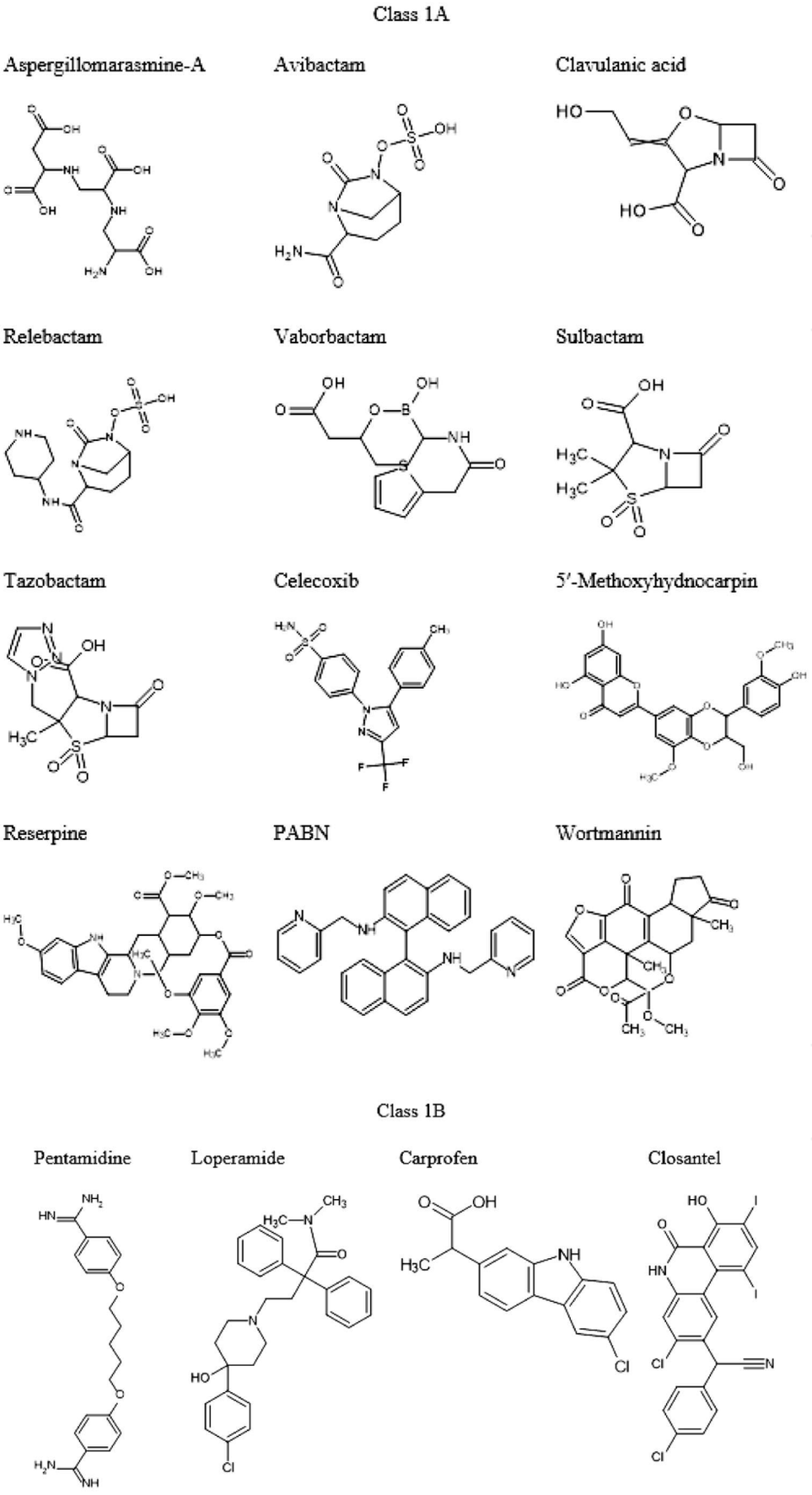

Figure 2.
Chemical structures of some antibiotic resistance breakers of class I.

enzymes act. These enzymes cause the detoxification of the antibiotic by exchanging the functional group of antibiotics, hydrolytic cleavage of a susceptible bond, founding the antibiotic substance, and by other oxidation-reduction reactions. An approach to inhibit these enzymes appears to be very effective in this regard. Several chemicals are employed to inhibit these modifying enzymes that are responsible for the degradation and alteration of antibiotic molecules. The two mostly employed groups of enzyme inhibitors are β-lactamase inhibitors and aminoglycoside enzyme inhibitors [2, 7].

2.2.1.1 β-Lactamase inhibitors (BLI)

BLIs help in overcoming resistance by deactivating the molecular mechanism of beta-lactamases, which hydrolyze the beta-lactam ring of drugs. These lactamases are classified into four classes depending on the similarities in their protein structure. Among these four, class B are metallo-β-lactamases while others, i.e., classes A, C, and D, are serine-β-lactamases [16]. The ARBs belonging to this group work by interfering with the penicillin-binding proteins. Several BLIs are employed including clavulanic acid, co-triclay, sulbactum, and tazobactum [2, 5, 17]. The description of some BLIs with their modes of action is given in **Table 1**.

BLI	Class	Mode of action	Drugs used for combinatorial therapy	Reference
Clavulanic acid	Differs from penicillin for the having oxazolidine ring	Stops the enzymatic reaction of β-lactamase by binding to its active site and enhances the antibacterial effect of the drug	Amoxicillin and ticarcillin	[18, 19]
Sulbactam	Semi-synthetic β-lactamase inhibitor	Irreversible binding to the active site of β-lactamase and inhibits the antibiotic breakdown	Ampicillin and cefoperazone	[18, 19]
Tazobactam	Penicillanic acids	Irreversible attachment to active site of β-lactamase	Piperacillin and ceftolozane	[18, 19]
Avibactam	Azabicycloalkanes	Protect the hydrolysis of β-lactam ring by lactamase by destructing the avibactam ring	Ceftazidime and meropenem	[18, 19]
Relebactam	Diazabicyclooctane	Produces a positive charge at pH 7 to decrease the efflux of inhibitors from bacterial strain leading to enhance antibacterial effect	Imipenem and cilastatin	[18, 19]

BLI	Class	Mode of action	Drugs used for combinatorial therapy	Reference
Nacubactam	Diazabicyclooctane	Greater affinity to selectively bind with penicillin-binding protein 2 (PBP2) as well as inhibition of β-lactamase	Intravenously used meropenem	[20]
Zidebactam	Bicyclo-acyl hydrazide	Greater affinity to selectively bind with penicillin-binding protein 2 (PBP2) as well as inhibition of β-lactamase	Parenteral of cefepime	[21]

Table 1.
Beta-lactamase inhibitors with their classification, mode of action, and combinatorial therapy.

2.2.1.2 Aminoglycoside-modifying enzyme inhibitors

Aminoglycoside-modifying enzymes (AME) [7] are classified into three types depending on the reaction they catalyze. These include N-acetyltransferases (AACs), O-nucleotidyltransferases (ANTs), and O-phosphotransferases (APHs) [2, 22]. The resistance caused by the alteration of antibiotics by these enzymes can be overcome by employing inhibitors in combination with the resistant antibiotic. The inhibitors target the modifying enzymes and allow the antibiotic to bind to the active site. These inhibitors which mimic the structure and charge of aminoglycosides have negatively charged binding sites but have a net positively charged molecule. It had been observed that the antibacterial effect of the drug streptomycin on O-nucleotidyltransferases was recovered by the synergic use of streptidine and streptomycin [23]. The study conducted by [22] demonstrated the potential of pyrimidinyl indole derivatives to be employed as AME inhibitors.

2.2.2 Inhibition of an efflux pump

Efflux pumps can intrinsically cause bacterial resistance to reduce the drug concentration by pumping it out. To overcome this problem, several approaches have been carried out. These approaches include repressing the genes responsible for encoding the proteins of the efflux pumps, avoiding the assembly of transporters of the efflux pumps, reducing the mechanisms that are responsible to give the energy required for the functioning of these efflux pumps, modifying the antibiotic to make it unrecognizable by pumps and obstructing the active site by making the antibiotic unable to bind [5]. Among these approaches, studies showed that the use of inhibitors that directly target the efflux pump has been proven to be very useful [2]. Some commonly used efflux pump inhibitors have been given in **Table 2**.

2.2.3 Permeabilization of membrane

The effective perforation of the antibiotic through the plasma membrane is responsible for its enhanced action. During bacterial resistance, the permeability of

Efflux pump inhibitor	Source	Reference
Catechin gallates	Extract of green tea	[24]
Abietane diterpines	*Rosmarinus officinalis*	[25]
Methoxylated flavones and isoflavones	*Thymus vulgaris*	[2]
Bonducellin	*Caesalpinia digyna*	[2, 26]
Compounds EA-371α and EA-371δ	Streptomyces fermentation extracts	[2, 27]

Table 2.
Commonly used efflux pump inhibitors.

the cell membrane for antibiotics declines which can be controlled by employing the synergistic use of antibiotics along with membrane permeabilizers [5]. For instance, the synergic use of azithromycin and colistin has been observed to be effective against resistant Gram-negative bacteria. These compounds, being cationic or amphipathic in nature, are capable of chelating the anionic lipopolysaccharides present in the complex structure of the outer membrane. As a result of chelation, the structure of the membrane is disrupted resulting in enhanced permeability for the drug [2]. The membrane permeabilizers that are mostly employed are aminoglycosides, polyamines, peptidomimetics, phenylalanine-arginine β-naphthylamide (PaβN), polymyxin B, colistin, ethylenediaminetetraacetic acid, cationic cholic acid derivatives, and plant-derived phenolic compounds [5, 28].

3. Nano-technology to counter antimicrobial resistance

Antibiotic treatment is the best prevailing approach for handling bacterial toxicity. Nevertheless, antimicrobial resistance action has developed as one of the principal dangers to community strength globally due to the overuse and mishandling of antibiotics. Although, the speedy discovery of novel antibiotics is mainly time-dependent associated with the development of antibacterial activity. The world is on the threshold of the post-antibiotic time. Beside these, efforts are being carried out to reduce antibiotic resistance by limiting the systematic exposure of the drug through a local antimicrobial treatment approach. For this purpose, several medical devices and biomaterials have been developed for the administration and monitoring of drugs locally. But this approach also faces some challenging issues including diffusion barriers within the local environment that prohibit drug molecules from reaching bacteria, different drug clearance mechanisms after administration render local application impractical or ineffective, and drug resistance developed by target bacteria reduces the therapeutic effectiveness of an antibiotic [29]. These challenges demonstrate the need for ongoing research into novel and efficient antibacterial techniques.

Modern developments in nanotechnology, especially the creation of drug-delivery nanoparticles, had a significant impact on medicine and healthcare [30]. Studies have revealed that nanomaterials are potentially able to overcome the antibacterial resistance of conventional antibiotics by employing various ways such as improving pharmacokinetics, uptake of antibiotics effectively inside the cell, interfering with bacterial metabolism, enhancing biofilm penetration, and altering biofilm microenvironments. The amalgamation of nanotechnology and antibiotics is the best auspicious approach to handle antibiotic-resistant microbes [31].

3.1 Nano-antibiotics

Nano-antibiotics are nanomaterials that either exhibit antibacterial activity on their own or increase the efficiency and safety of antibiotic treatment [32]. The capacity of nano-antibiotics to control infections *in vitro* and *in vivo* has been investigated and proved in several studies [33]. Antimicrobial nanoparticles (NPs) may not have direct and immediate side effects, in contrast to many antimicrobial drugs now utilized in clinical settings, yet it is debatable whether they may be hazardous over the long-term use. Most remarkably, resistance to NPs' antimicrobial activity would need several concurrent changes since antimicrobial NPs target various biological pathways prevalent in a variety of microbial species. When compared to the synthesis of antibiotics, the preparation of antimicrobial NPs might be more affordable, and they are fairly stable enough to be kept in storage for an extended period of time [34]. Furthermore, certain NPs are resistant to extreme circumstances such as sterilization at high temperatures that render traditional antibiotics ineffective. Multiple benefits come with antibiotic delivery employing nanomaterials, including manageable and uniform delivery in the targeted tissue, enhanced solubility, sustained and controlled release, enhanced patient compliance, reduced negative effects, and improved cellular internalization [32, 35].

Effective antibacterial activities of different nanomaterials are considered influenced by their high surface area-to-volume ratios and distinctive physical and chemical properties [36]. Furthermore, recent research demonstrated that bacteria present in nature do not become resistant to antibacterial drugs [37]. The mechanisms carried by nanomaterials to perform antibacterial activity include photo-catalytic products of reactive oxygen species (ROS) that harm viral and cellular components, compromise the plasma membrane and cell wall of bacteria, disruption of energy transduction, and interfere with the enzyme activity and synthesis of DNA [38, 39]. Various antimicrobial mechanisms of nanomaterials have been shown in **Figure 3**.

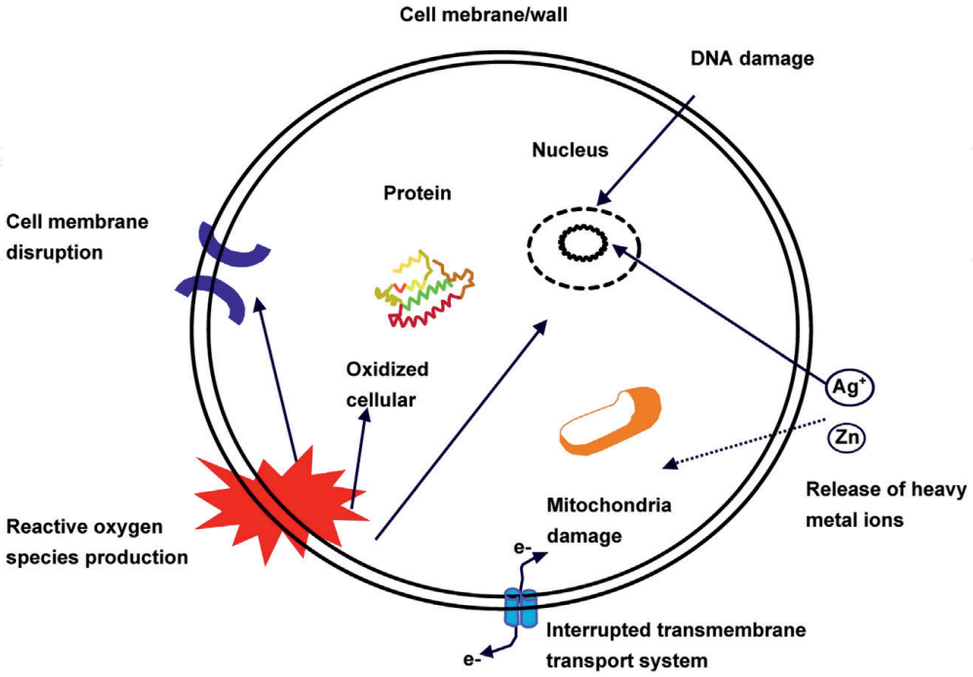

Figure 3.
Various antimicrobial mechanisms of nanomaterials.

The efficacy of local antimicrobial treatment has also been enhanced by the nanoparticle-based delivery approaches by targeting three aspects including encouraging the localization of the drug to the microbes, controlling the interaction between drug and microbes to avoid antibacterial resistance, and allowing drug-free anti-virulence therapy [29]. Antimicrobial mechanisms of inorganic nanomaterials, their possible applications in medicine and industry, and other details are summarized in **Table 3**.

Researchers investigated using nanoengineered systems to encapsulate antibiotics as a single or numerous antibiotic delivery systems. These delivery systems are membrane-bound nanoparticles, polymeric nanoparticles, solid metallic nanoparticles, lipid polymer hybrid nanoparticles, metal oxide nanoparticles, mesoporous nanoparticles, carbon-based nanoparticles, chitosan nanoparticles, etc. Among them

Nanomaterial	Antimicrobial mechanism	Clinical and industrial applications	Reference
Ag NPs	Release silver ions which destroy the integrity of the plasma membrane and electron transport chain derangement as well as damage the bacterial DNA	Employed in the dressing of wounds of diabetic foot disease and surgical wounds, coatings for medical devices, portable water filters, antibacterial agents and antifungal agents	[40]
ZnO NPs	Accumulate nanoparticles inside the cell causing the plasma membrane disruption by synthesizing hydrogen peroxide and releasing zinc ions	Antibiotic creams, lotions, and ointment, surface coating of medical devices, mouthwash	[40]
TiO2 NPs	Synthesis of reactive oxygen species leading to disruption of plasma membrane and cell wall	Antibacterial agent, food sterilizing agent, air purifiers, water treatment systems	[40]
Au NPs	Binding with plasma membrane through strong electrostatic bond	Photothermal therapy with near-infrared light, adjuvant treatment after serious infections antibacterial agent, antifungal agent	[41]
Chitosan	Trace elements chelate the plasma membrane resulting in enhancing the plasma membrane permeability and disruption as well as inactivating the enzymes	Drinking water disinfectants, bacteria immobilizer, bactericidal activity in biomedical products	[41]
Fullerenes	Damage the integrity of the plasma membrane and improving the activity of infiltrating neutrophils	Potential disinfection applications	[42–44]
CNTs	ROSs disrupt the plasma membrane through protein and lipid peroxidation	Antibacterial agent, biofouling-resistant membranes, water filter, surface-coating	[45, 46]
NO-releasing NPs	Release nitric oxide as well as synthesize ROSs	Infected wound and diabetic foot treatment	[47]
Nanoemulsion	Disrupting membrane permeability disruption of the spore-coat	Antimicrobial inhaler, anti-biofilm agent, nasal application vaccine delivery agents	[48]

Table 3.
Antimicrobial nanomaterials with their mechanism.

and all other nanomaterials, polymeric, membrane-bound, and mesoporous delivery systems are the most studied [32]. In this chapter, we have discussed different types of nanoparticles to roughly classify them into organic and inorganic nanoparticles.

3.2 Inorganic nanoparticles

Inorganic nanoparticles have a broad range of applications in the field of medical science because of their cytotoxic, genotoxic, and carcinogenic properties [49]. Inorganic NPs are classified as metallic and metal oxide nanoparticles, which have been studied widely for their antimicrobial characteristics. Reactive oxygen species (ROS) production is one of the primary antibacterial processes used by both metal and metal oxide nanoparticles [50]. Recently, bacterial cells and enzymes have been investigated as a potential living and environmentally pleasant technique for synthesizing nanoparticles for the internal or extracellular synthesis of metal-based nanoparticles, e.g., silver, gold, and cadmium sulfide nanoparticles [39].

3.2.1 Metallic nanoparticles

A metallic nanoparticle (MNP) is defined as a three-dimensional nanosized metal with a size range from 1 to 100 nm. These types of nanoparticles can be produced using various chemical functional groups and combined with ligands, drugs, and antibodies. The characteristic feature of a nanoparticle is the surface plasmon resonance and optical quality [51]. The precious metals, especially silver and gold, have attracted a lot of interest in a variety of fields of science and technology including catalysts, photography, and the medical field as potential antitumor and antimicrobial agents [50]. The production of ROS, cation release, biomolecule damage, adenosine triphosphate (ATP) loss, and membrane interaction are the main components of the mechanisms of metal nanoparticles' antibacterial behavior [52].

3.2.2 Metal oxide nanoparticles

Metal oxide nanoparticles also range from 1 to 100 nm in size with unique electronic characteristics. Numerous metal oxide nanoparticles exhibit an antimicrobial impact, either independently or in response to exposure to light [53]. Some examples of the most widely studied nanoparticles include titanium oxide (TiO_2), iron oxide (Fe_2O_3), zinc oxide (ZnO), copper oxide (CuO), manganese oxide (MnO_2), zirconium oxide (ZrO_2), aluminum oxide (Al_2O_3), and silica oxide (SiO_2) [50]. Among all these, a detailed investigation of titanium oxide has been carried out for possessing photocatalytic activity. The exposure to UV rays induces the photocatalytic activity of TiO_2, followed by the production of hydroxyl free radicals, which damages the important components of bacterial cells such as proteins, lipids, polysaccharides, and DNA by causing their oxidation. This way, TiO_2 and other metallic oxides exhibit their antimicrobial effect [54]. Despite all these, concerns have been raised about zinc oxide, tri-manganese tetroxide, magnetite, and magnesium oxide nanoparticles. Therefore, it is crucial to check and make sure the metal oxide nanoparticles, which are employed in medical applications [55].

3.3 Organic nanoparticles

Antimicrobial medications packed in organic nanoparticles have recently emerged as possible strategies for improving the therapeutic effects and minimizing adverse

drug reactions. Organic nanoparticles could provide long-term durability and biocompatibility [56]. By releasing antimicrobial peptides or other antimicrobial agents, polymeric nanoparticles with cationic surfaces such as quaternary ammonium compounds, quaternary phosphonium, or alkyl pyridinium destroy microbes. Studies demonstrated that higher positive charge levels can cause antimicrobial effects through an ion-exchange mechanism between the bacterial membrane and the charged surface [50]. However, some organic nanoparticles that are being employed in the medical field, especially for antimicrobial effects, are discussed in detail below:

3.3.1 Fullerenes (C60) and fullerene derivatives

Recent research on the antibacterial effects of fullerenes is based on a paucity of information. Despite being essentially insoluble in aqueous environments in their natural state, fullerenes may be dispersed in water using a number of newly developed techniques. The production of stable colloidal C60 aggregates (nC60) in water *via* several methods, has shown their remarkably powerful and widespread antibacterial action [42]. Photo-catalytic ROS generation in eukaryotic cells is a part of the controversial antibacterial mechanism of nC60. According to several investigations, lipid peroxidation in the cell membrane is the mechanism through which nC60's antibacterial action against prokaryotic cells operates [43].

The lack of protein oxidation and antibacterial characteristics of nC60 that are independent of light and oxygen point to a ROS-independent toxicity mechanism. Recent findings imply that solvent pollutants such as tetrahydrofuran (THF) or its oxidative byproducts that are utilized or produced during C60 synthesis might be the cause of the reported poisonousness of nC60 in both microbes and human cells [44]. In particular, after irradiation, the harmless nature of the nC60 suspension made using THF-independent procedures was established. Additionally, nC60 produced without the use of any biological solvent caused neither acute nor subacute toxicity in animals and protected livers in a dose-dependent way against free-radical damage. In addition to the cell wall, the subsequent destruction of the plasma membrane structure is how the carboxy fullerene exerts its antibacterial effect. Similar to vancomycin's antibacterial properties, the alkylated derivatives of C60-bis (N,N-dimethyl-pyrrolidinium iodide) influence the electron transport chain and efficiently suppress bacterial progression. Fullerols, also known as polyhydroxylated fullerenes [C60(OH)n] [57], showed high antibacterial action against a variety of pathogens while being less hazardous than nC60 [45].

The blood-ocular barriers can be avoided using fullerol as a medication carrier. Although fullerols have modest acute toxicity, their maintenance in the body has raised questions regarding potentially prolonged harmful consequences. The physicochemical characteristics of the aggregated form of C60 in water, e.g., fullerene water suspensions (FWS), differ from that of bulk solid C60, including antibacterial action [58]. For instance, FWS produced by employing (THF) tetrahydroflurin as a solvent (THF/nC60), sonicating C60 soluble in toluene with H_2O (son/nC60), agitating C60 powder in H_2O (aq/nC60), and adding polyvinylpyrrolidone (PVP/C60) as a solubilizer had effective antibacterial action [42].

3.3.2 Carbon nanotubes (CNTs)

The characteristic electrical, mechanical, optical, and thermal capabilities of carbon nanotubes, which are tube-shaped nanostructures consisting of pure carbon

atoms tightly bound in hexagonal arrays, have attracted a lot of attention. Although multiwalled nanotubes (MWNTs) include numerous nested tubes with sizes ranging from one hundred nanometers to several tens of micrometers, single-walled nanotubes (SWNTs) have a particular pipe with a length in the series of 1–5 nm [32, 44]. The cytotoxicity of carbon nanotubes in a macrophage is extremely high and in the order of SWNTs > NMWNTs > Nquartz > NC60. When SWNTs were injected *in vivo*, there were additional reports of temporary inflammation and lung damage. The reduced water dispersal of clean CNTs weakened the potential of SWNTs as an antimicrobial agent, despite the suggestion that they had antimicrobial capabilities. Recent studies have shown that stabilizing CNTs with surfactants such as sodium dodecyl benzene sulfate [SDBS], Triton-X, and PVP can significantly increase their water discrepancy [32, 59].

Single-walled nanotubes (SWNTs) have the highest antibacterial action among other carbon-related nanoparticles, which are often cytotoxic by combining oxidative stress potentially in a synergistic manner [46]. In a recent study, specific three-step antibacterial processes of SWNTs were proposed. First, SWNT bacterial interaction was developed followed by membrane disruption, and then oxidation of the membrane was done in a way depending on the electronic arrangement. According to [60], SWNTs may effectively inhibit the formation of biofilms and the consequent biofouling of surfaces (e.g., water filtration membranes). SWNTs are effective antimicrobial biomaterials due to their extraordinary chemical strength and the simplicity of their function. The point of accretion, the stabilizing the special effects of the usual carbon-based substances, and the bioavailability of CNTs are taken into account for the antibacterial capabilities. For instance, at the same concentrations, well-dispersed CNTs are less cytotoxic than rope-like CNT agglomerates. Using CNTs for water filtration, efficient poliovirus and *Escherichia coli* inactivation, and MS2 bacteriophage elimination has received more attention recently. Contrary to traditional filters, CNT filters allow for repeated cleaning to restore their full filtering effectiveness [61]. Delivering CNT nanoclusters to a region that is contaminated, monitored by natural microbial adsorption to the collections and the death of drug-resistant microbes upon near ultraviolet radioactivity is another way that CNTs may be employed for antimicrobial photothermal treatment [62]. The possible mechanisms of action of metallic nanoparticles that cause cell death of Gram-negative bacteria are shown in **Figure 4**. The figure illustrates how nanoparticles disrupt the cell structure. They interact with the cell membrane, disrupt the membrane integrity and after entering into the cell, cause the generation of reactive oxygen species by antioxidant depletion, damage DNA and proteins, interfere with nutrient assimilation and electron transport chain, altering the cell signaling process by dephosphorylating the peptides present on the tyrosine residue and ultimately cause the inhibition of singling pathway responsible for the growth of the bacterial cell.

3.3.3 Liposomal nanoparticles

The spherical lipid containers known as liposomes have a layered membrane structure made of natural or synthetic lipid molecules that are amphiphilic in nature. Their capacity to contain hydrophilic drugs inside the aqueous compartment and/or hydrophobic drugs inside the lipid bilayer sets them apart from other nanoparticles [63]. Since 1995, when the Food and Drug Administration (FDA) approved the major liposomal medication, Doxil (doxorubicin encapsulating PEGylated

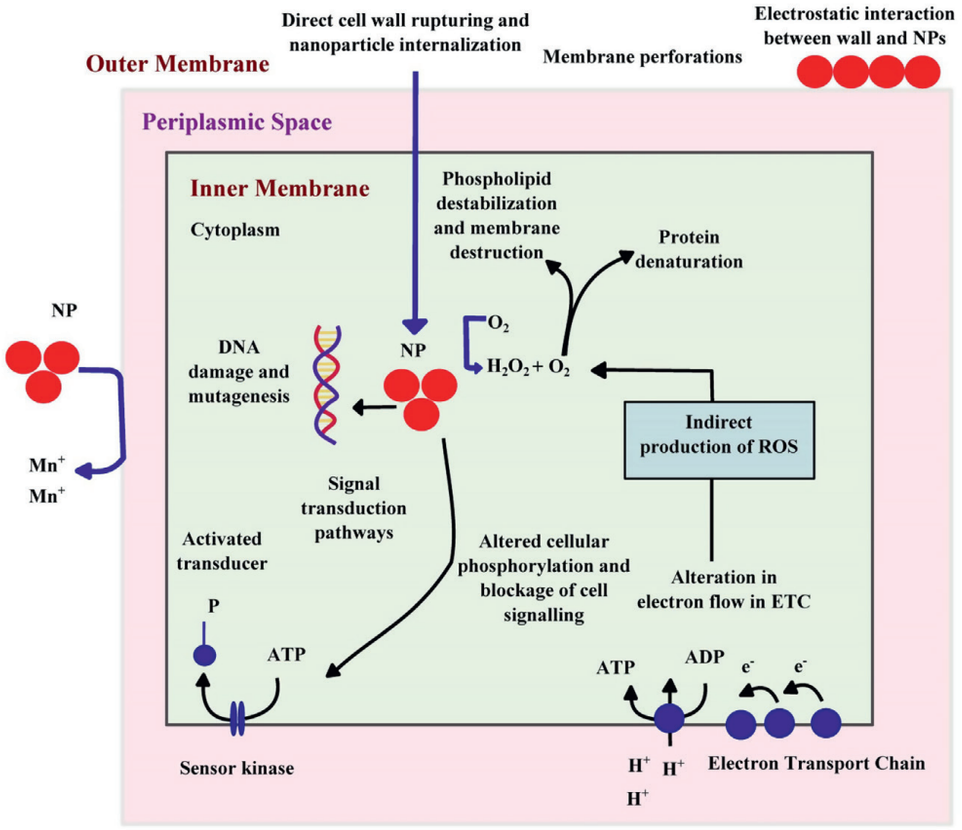

Figure 4.
Possible mechanisms of action of metallic nanoparticles causing Gram negative bacterial cell death [39].

liposomes), liposomes have been considered as promising and clinically acceptable delivery vehicles for a variety of antimicrobial diseases [64, 65]. Liposomes are the most widely employed antimicrobial medication carrier system due to their plasma membrane-like phospholipid bilayer structure and ability to fuse with pathogenic microorganisms [35]. Numerous studies have demonstrated that liposomal encapsulation enhances the stability of antibiotics by reducing their interaction with plasma proteins, decreasing recognition by macrophages, and providing safety by safeguarding them from chemical or immunological deactivation and unwanted enzymatic degradation. All these result in improved pharmacodynamic and pharmacokinetic profiles by extending the bloodstream's circulation time and making the targeted administration of an antibacterial drug to the specific sites of infection possible *via* various routes [66]. Additionally, the antibiotic medication may be enclosed and reserved in phospholipids bilayers and in the aqueous core individually without undergoing any biochemical alterations. Another unique property of liposomes is that the surface modification of these particles with stealth materials helps to improve their *in vivo* stability and to allow targeted delivery of liposomes. When using liposomes for antimicrobial drug delivery, a number of factors should be taken into account, including the physical and chemical characteristics of lipids, particle mass, and poly-disparity, surface charge, shelf-life stability, and re-reducibility and possibility for large-scale manufacturing [67]. The applications of some antibiotics enclosed in liposomes are given in **Table 4**.

Drug	Liposomal formulation	Clinical applications	Reference
Amphotericin B	AmBisome (hydrogenated soy, phosphatidylcholine, cholesterol, and distearoylphosphatidylglycerol)	Treat *Aspergillus* spp., *Candida* spp., *Fusarium* spp., and other fungal infections in methylmalonic acidaemia, neutropenic visceral leishmaniasis patients	[68]
Polymyxin B	1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol	Treating *Pseudomonas aeruginosa* related infections (e.g., pneumonias and chronic bronchopneumonia's of cystic fibrosis)	[69]
Ampicillin	Soybean phosphatidylcholine [16] and cholesterol	Elevated drug stability and higher antimicrobial activity against *Salmonella typhimurium*	[70]
Benzyl penicillin	Dipalmitoyl-phosphatidylcholine (DPPC), cholesterol, and dimethylammonium ethane carbamoyl cholesterol (DC-chol)	Inhibition of penicillin-sensitive strain of *Staphylococcus aureus*	[71]
Ciprofloxacin	Dipalmitoyl-phosphatidylcholine, dipalmitoyl-phosphatidylglycerol, and cholesterol	Inhibition *Salmonella dublin* in mouse spleen	[72]
Vancomycin and teicoplanin	Egg phosphatidylcholine, diacetylphosphate, and cholesterol	Show enhanced bactericidal activity against methicillin-resistant *Staphylococcus aureus*	[73, 74]

Table 4.
Clinical applications of some antibiotics enclosed in liposomes.

4. Conclusion

As bacterial resistance is an ever-increasing phenomenon and the spectrum of antibiotic action is reducing due to the emergence of resistant strains, there is a dire need to discover novel approaches to combat this alarming issue. Synergic use of antibiotic resistance breakers helped a lot in this regard. The development of new ways to treat bacterial resistance is time taking and unavailability of resources is the major hindrance. However, the utilization of nanotechnological approaches in the medication and drug delivery system plays an important role in effectively delivering the drugs to their targeted areas and it seems to be a promising approach in the future. A lot of work is being carried out in this field and researchers have been working to devise more ways to tackle the bacterial resistance.

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