Chapter 6
Limiting Antibiotic-Resistant Bacteria Using Multifunctional Nanomaterials

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Abstract In the current scenario, antibiotic-resistant bacteria become a global threat to human health, and it has been predicted that by the year 2050, death caused due to bacterial infection will surpass the cancer-related death. Existing antibiotic therapy experiences several limitations like side effects, poor stability, and solubility which leads to its inefficiency in antimicrobial therapy. To overcome these limitations, research has been focused on alternative strategies like use of nanomaterials in the formulation of antimicrobial agents due to advantages like drug-targeting ability, biodistribution, enhanced uptake, and favored physicochemical properties. Nanomaterials interact with the cellular component of microbes, and their antimicrobial behavior depends majorly on surface chemistry, size, shape, and core material. This chapter elaborates on the drug-resistant mechanism of microbes as well as the role of nanomaterials (nitric oxide-releasing, chitosan-based, and metallic) in combating drug resistance. Various bacterial-based diseases in animals are also liable to be transferred in humans and cause serious illness. The potential of nanomaterials in the prevention and treatment of diseases in animal models is also the highlight in the present article. Finally, we also discussed the clinical approaches of nanoformulation in combating drug-resistant microbes.

Keywords Multidrug resistance · Nanomaterials · Antimicrobial · Biofilm · Animal disease · Clinical trials · Antimicrobial peptides · Antitoxins

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V. Kumar et al. (eds.), *Nano-Strategies for Addressing Antimicrobial Resistance*,
Nanotechnology in the Life Sciences, https://doi.org/10.1007/978-3-031-10220-2_6

6.1 Introduction

Multidrug-resistant (MDR) bacteria remain a key challenge for the treatment of bacteria-driven life-threatening diseases. According to an estimate, by the year 2050, the deaths caused by bacteria-driven diseases may surpass the mortality caused by cancer. Currently, several bacterial species are reported to be resistant to the essential drugs like methicillin, carbapenem, and vancomycin, thus producing methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Enterobacteriaceae*, and vancomycin-resistant *Enterococcus*, respectively (Ssekatawa et al. 2020; Willyard 2017). Some of the common reasons for drug resistance are irrational and overuse of antibiotics, bacterial adaptation to biofilms formation, and prolonged use of antibiotics for the treatment of bacterial diseases (Munir et al. 2020). Excess use of antibiotics causes selective pressure on microbes which in turn develop the genes encoding antibiotic resistance and thus produce new strains in which the resistance is transferred via horizontal or vertical transmission (Arzanlou et al. 2017a).

Antibiotics inhibit the growth of bacteria by different mechanisms including inhibition of cell wall synthesis and hindering DNA, RNA, protein synthesis, and biofilm formation. MecA genes in bacterial cells are reported to impart resistance against antibiotics such as penicillin and methicillin (Berger-Bachi 1994). Single microbe-like superbugs can acquire MDR by adapting drug-resistant genes from other bacteria. The enzyme, such as New Delhi metallo-b-lactamase-1 (NDM-1), can degrade the β-lactam ring, thus making a range of antibiotics ineffective against the bacterial strain (Rolain et al. 2010). *Mycobacterium tuberculosis* and *S. aureus* are other common examples of drug-resistant bacteria causing serious concern and threat to the global healthcare community (Munir et al. 2020). Thus, the origin of antibiotic-resistant pathogenic bacterial strains requires immediate attention, and some novel approaches are required to inhibit their growth and transmission. In this context, nanotechnology-based novel therapeutic strategies have shown promising results in controlling the growth of MDR microbes.

To overcome the antibiotic-resistant complications, it is important to understand the mechanism by which microbes escape the traditional antibiotic therapy. There are two types of bacterial growth: (i) planktonic growth, described as unicellular, free-swimming microbes not attached to a surface, and (ii) biofilm growth phase, characterized as multicellular sessile state which forms communities (Berlanga and Guerrero 2016).

Biofilm formation is an advanced method that allows bacteria to survive in harsh circumstances by developing permanent colonies with great ability to dissociate and form new colonies (Rizzato et al. 2019; Majumdar and Pal 2017). Bacterial biofilms are made up of a dense and hydrated clump of bacteria that are attached to a surface and are encased in a dense external matrix of exopolysaccharides, extracellular deoxyribonucleic acid (DNA), and amino acids (Blair et al. 2008). While biofilm formation in the common bacteria like *Staphylococcus epidermis* and *Pseudomonas aeruginosa* is well-known to protect them from various antibiotics, diverse other

biofilm-forming microbes also exist which confer resistance against wide range of antibiotics. For example, yeast *candida albicans* and obligate anaerobe *Porphyromonas gingivalis*, when grown in the biofilm, have been reported to be less susceptible to antibiotics in comparison to free-floating cells (Stewart 2002). Human lung, urethra, colon, ear infections, infective endocarditis, gum infection, and wound-related infections are linked to biofilms formation (Valappil 2018). In comparison to planktonic bacterial growth, biofilms are thought to be ~1000 times more resistant to antibiotics (Rossi-Fedele, Roberts 2007). Biofilm bacteria are subjected to cell density-dependent control from their extracellular polymeric substances (EPS) matrix, and thus as a result of high density, they are discharged into the surroundings as free-floating bacteria. Furthermore, both biofilms and host immune responses enhance the transformation of normal nonpathogenic commensal bacteria into virulent forms in the human body (Marsich et al. 2012). The evolution of survival mechanisms has been aided by the increased genetic mutation rates within biofilms. The expression of certain efflux pumps and upregulation of various proteins could cause diffusion across the biofilm. In this context, deletion of genes encoding the biofilm-specific efflux pump, PA1874–1877, confers the *P. aeruginosa* sensitivity to antibiotics like gentamicin and ciprofloxacin. These genes are not found to be overexpressed in planktonic cells proving their importance in biofilm resistance. Furthermore, increased production of toxin-antitoxin modules inhibits important cell operations like translation (Zhang and Mah 2008; Eleraky et al. 2020). Thus, due to the diversity and anonymity of biofilm-resistant processes, innovative nanosystems are envisaged to effectively inhibit the spread of resistant bacterial strains.

Nanoparticles (NPs) offer multifunctional aspects of eradicating MDR microbes because of their ability to act as transporters for common antibiotics as well as natural antibacterial substances (Wang et al. 2017c). The most widely used aspect of nanomaterials (NMs)-based drug delivery system is its ability to introduce a diverse array of therapies being linked to or confined inside their huge surface area and controlled rate of targeted delivery to infected site (Gholipourmalekabadi et al. 2017; Baptista et al. 2018). NMs-mediated delivery can improve the therapeutic index and pharmacokinetic profile of encapsulated drugs in comparison to free drugs which leads to decrease in the required dose to achieve an equivalent clinical effect. This will reduce the adverse toxic side effect caused due to high and frequent dose administration (Gao et al. 2018). Various NPs are reported to be used as efficient drug delivery agents, i.e., liposome, polymeric NPs, inorganic NPs, dendrimers, etc. Rinaldi et al. demonstrated the rifampicin-loaded liposome (Rif-Lipo) for the treatment of pulmonary infection caused due to *Mycobacterium abscessus* (Rinaldi et al. 2021). Synthesized nanoformulation was found to be stable at room temperature and 4 °C for 90 days. The authors showed that 18 h exposure of 96 μM Rif-Lipo nanoformulation inhibits the *M. abscessus* infection with a similar effect of 192 μM rifampicin alone. Targeted delivery of drugs and antibiotics can also be achieved efficiently by using various NPs. In this context, Güncüm et al. demonstrated the antibacterial activity of polymeric (poly(vinyl alcohol)/sodium alginate) NPs containing amoxicillin (poly-AmoNPs) against *Escherichia coli (E. coli)* and

S. aureus (Güncüm et al. 2018). The result showed that with a decrease in pH, the release of amoxicillin also decreases which induces the controlled release of drug at infectious site. This formulation has a similar effect as a free drug against *E. coli* and *S. aureus*. Further, Wang et al. synthesized gold-silver nanocage coated with the pattern recognition receptors (PRRs) (found in macrophage membrane) (Shi et al. 2018). Macrophages were treated with *S. aureus* and *E. coli* to confirm the expression of pathogen-related receptors on their membrane surface. This formulation promotes the adherence of NPs to specific bacteria for targeted therapy and delivery of the drug. Also, the gold-silver nanocages can convert NIR (laser light) into heat to destroy the bacteria by using laser irradiation treatment. The hollow structure of nanocage can be well utilized for the encapsulation of drugs for the targeted therapy.

6.2 Mechanism Underlying Antibiotic Resistance in Microbes

Existing antibiotics are reported to inhibit the growth of microbes via affecting three different machineries: DNA replication, translation process and cell wall synthesis. Interestingly, bacterial cells evolve various strategies to counter the inhibitory functions of antibiotics by developing resistance mediated by mutations in chromosomes. Non-resistant bacteria get eradicated by antibiotics, whereas resistant species survive the exposure and eventually transfer the resistance mechanism to next generation through horizontal or vertical transfer (Arzanlou et al. 2017b; Ruddaraju et al. 2020). Various mechanisms of antibiotic resistance in microbes are discussed below in detail and summarized in Fig. 6.1.

6.2.1 Competition to Antibiotics and Resistance to Persister

Bacterial cells can produce molecules displaying competitive inhibition for each antibiotic to acquire resistance against the antibiotics. In this context, sulfonamide-resistant bacterial cells produce a high amount of pare-aminobenzoic acid (PABA) to confer resistance. Sulfonamides hinder bacterial nucleic acid synthesis by inhibiting the bacterial enzyme dihydropteroate synthetase (DHPs) in the folic acid pathway. In *Neisseria meningitidis* and *S. aureus*, PABA competes with the sulfonamides for enzyme DHPs and leads to resistance in bacterial cells (Ponce et al. 2017).

Inert persisters occur in the infected bacterial community causing a repetition of infection even after the treatment due to acquired resistance against antibacterial drugs. Persister cells decreased their metabolic rate by gene shift to achieve resistance against antibiotics. Bacterial community exposed to antibiotics showed that some of the populations are sensitive to drugs, while other remains unaffected

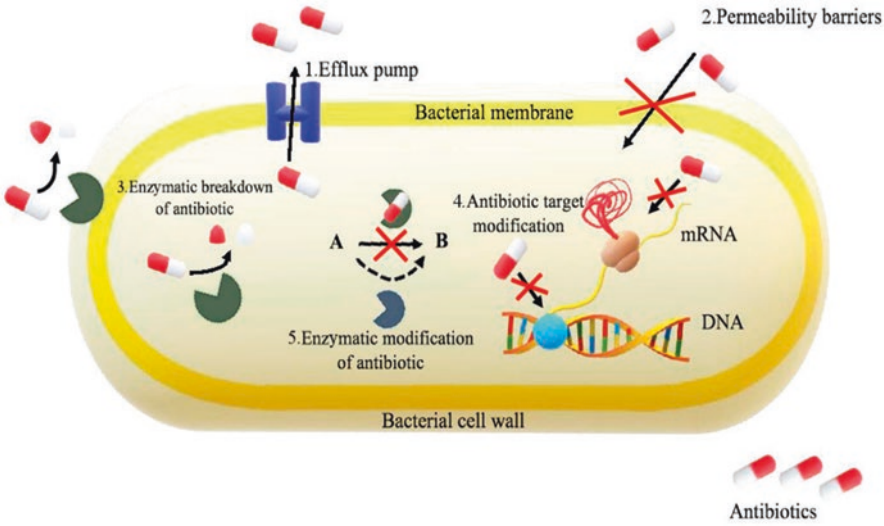

Fig. 6.1 Scheme showing mechanistic action of antimicrobial resistance (Pauter et al. 2020)

indicating the treatment completion for certain infection. In some cases, persisters shift to an active metabolic state again to cause reinfection.

6.2.2 Low Drug Uptake and High Efflux

Reduced rate of drug uptake and high efflux rate are two major mechanisms that simultaneously regulate antibiotic resistance. For example, *P. aeruginosa* low sensitivity for a drug could be due to the presence of inner membrane protein (H^+/drug antiporter protein) in the periplasmic space attached to a linker protein. Regulatory protein suppresses the gene encoding efflux protein; thus, mutation in regulating protein leads to overexpression of efflux protein and high MDR of *P. aeruginosa* (Nikaido 2009). In addition, energy driven by transmembrane protein could also be utilized by nine efflux pumps expressed in *E. coli* that facilitate the development of resistance in bacteria by expelling many antibiotics (Du et al. 2018).

Several genes are reported in both gram-negative and gram-positive bacteria that encode efflux pumps such as tetracycline efflux pump encoded by TetB, TetK, and TetA. Transfer of these genes to bacterial cells may be attributed to transposons and horizontal gene transfer on the plasmid (Blair et al. 2015). Most of the gram-negative bacteria are reported to be resistant against chloramphenicol and fluoroquinolones primarily due to the efflux effect. *Enterococcus faecalis* develop resistance against dalfopristin and quinupristin antibiotics by using the same efflux mechanism. Further, decreased uptake of antimicrobial drugs in gram-negative bacteria causes

resistance against aminoglycosides, whereas resistance to vancomycin may be attributed to the increased thickness of cell walls in microbes (Blair et al. 2015).

6.2.3 Biofilm Formation

Resistance in bacteria from several antibiotics may be attributed to the biofilm formation, which causes chronic infections. Biofilm-producing bacteria exhibit ~1000 times more resistance against antibiotics than bacterial species that do not form biofilms (Arciola et al. 2018). At the initial stage of biofilm formation, antibiotic treatment is found to be more effective because the microbial population is not completely adapted into the biofilm community (Munoz-Egea et al. 2016). During biofilm formation, EPS assembles to facilitate the localization of bacterial community; however, it acts as a diffusion barrier for antibiotics. Various mechanisms are suggested regarding EPS mediating antibiotic inhibition. Here, at the initial stage, the pore size of the matrix is small enough to hinder the entry of antibiotics. The negatively charged matrix further inhibits the effect of antibiotics on bacterial cells, and the enzymes located in EPS induce covalent modification in antibiotics that lead to the inhibition of their antimicrobial action (Ferreira et al. 2010). Additionally, EPS also acts as a barrier to nutrient and oxygen supply, thus promoting indirect resistance to bacteria against antibiotics. Bacterial cells located deep inside the biofilm exhibit lower metabolic rates due to less supply of nutrients and thus are also less susceptible to antibiotics.

The multicellular nature of biofilm is one of the key factors responsible for antibiotic resistance. EPS accumulates the bacterial cells together and develops the multicellular consortia, which forms a heterogeneous environment inside the biofilm and establishes a multicellular system. If the steps of multicellular structure formation of biofilm can be disrupted, antibiotic efficacy as well as host defense system could be improved. Another mechanism regarding the resistance of the biofilm community is the internalization of resistance genes by horizontal gene transfer via conjugation method (Mah 2012). Biofilm provides a compatible environment for gene transfer such as high genetic competence, accumulation of genetic elements, and high cell density (Fux et al. 2005). Several studies have reported that the conjugation process is more efficient in biofilms compared to planktonic cells (Van Meervenne et al. 2014; Sharma et al. 2019).

6.2.4 Antibiotic Modification

Several microorganisms express the drug-resistant genes, which encode the enzymes responsible for covalent modification of antibiotics such as aminoglycosides, tetracycline, quinolones and β-lactams (Laxminarayan et al. 2013). β-Ring of β-lactam has been reported to be hydrolyzed by β-lactamase enzyme leading to resistance in

β-lactamase-sensitive microbes. Horizontal transfer of β-lactamase gene on bacterial plasmids or decreased activity of a repressor protein that inhibits β-lactamase gene transcription is the major cause of resistance development in microbes (Moyá et al. 2012; Munir et al. 2020).

Recently, New Delhi metallo-β-lactamase 1 (NDM-1)-producing carbapenem-resistant microbes have been discovered. Several NDM-1-expressing bacteria are resistant against a majority of antibacterial drugs used to treat serious infections. Research on samples collected from various regions reports that they are tolerant to monobactam aztreonam, aminoglycosides, quinolones, tetracycline and β-lactam antibiotics (Kumarasamy et al. 2010). Moreover, the aminoglycoside resistance gene encodes enzymes causing a covalent modification of the OH and NH_2 group of aminoglycosides which leads to the decreased affinity with the 30S ribosomal subunit, thus inhibiting the antibacterial activity. Robicsek et al. reported the reduced susceptibility of clinical bacterial isolates against ciprofloxacin due to the expression of gene encoding aminoglycoside acetyltransferase. This enzyme inhibits ciprofloxacin's activity by N-acetylating the amino nitrogen on the piperazinyl group (Robicsek et al. 2006).

6.2.5 Swarming

Swarming represents social motility enabling differentiated bacterial cells to migrate. Swarming is similar to the biofilm community and characterized by a high level of resistance to antimicrobial treatments (Lai et al. 2009). Several swarming bacterial species like *P. aeruginosa*, *Salmonella enterica*, and *B. subtilis* are reported to exhibit multiple antibiotic resistance. Swarming bacteria exhibit three different strategies against antibiotics including high cell density, lower exposure to antibiotics due to circulation within the multilayer structure, and death of directly exposed individuals (Butler et al. 2010). Reports showed that even in the absence of swarming, high cell density promotes bacterial survival; however, movement ability, as well as the speed of movement, offers an extra advantage to swarm as an effective strategy against antimicrobials agents (Butler et al. 2010).

6.3 Nanotechnology-Mediated Strategies to Overcome MDR in Microbes

The emergence of new drug-resistant microbial species and the limited production of antimicrobial drugs have created a serious concern for human health. Synthesis of new antibiotics is a complex process and takes around 10–15 years for approval and also has a very high production cost (Eleraky et al. 2020). Therefore, as an alternative, people have looked at using NMs as an effective antimicrobial. NPs are

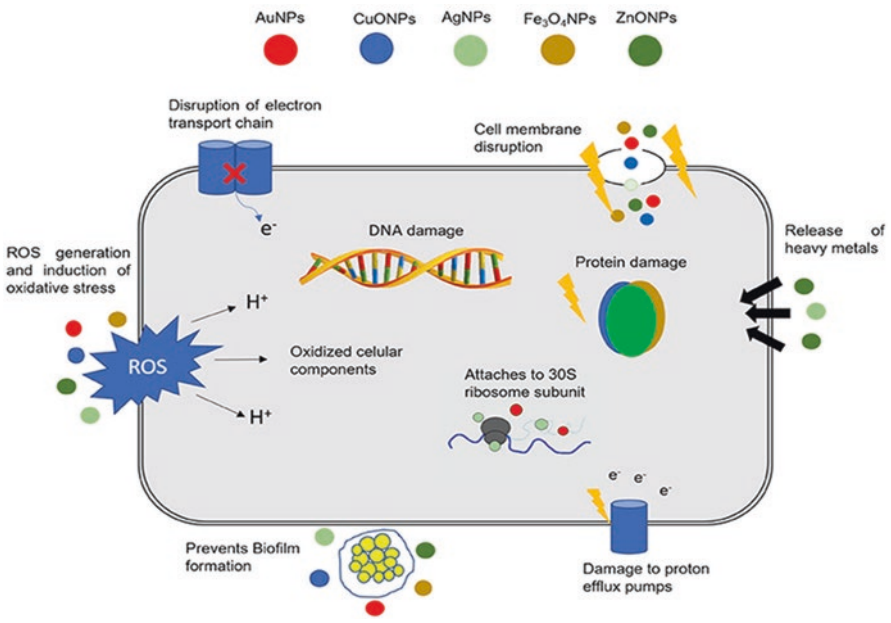

Fig. 6.2 NPs act on bacterial cells through different mechanisms (Baptista et al. 2018)

also reported to enhance the physicochemical property and stability of existing antibiotics, prolong their release, and facilitate targeted delivery to the infection site, with reduced side effects (Patra et al. 2018). NMs possess various mechanisms to overcome microbial drug resistance (Fig. 6.2), which can be governed by their physicochemical properties like size, surface charge, and solubility. Nitric oxide-releasing NMs, metal-based NMs, and chitosan-derived NMs are reported to prevent microbe resistance (Pelgrift and Friedman 2013). Encapsulation of antibiotics in NMs, inhibition of biofilm, increasing drug influx, and decreasing efflux are some of the mechanisms underlying their antimicrobial efficacy (Kaur et al. 2019; Tang and Zheng 2018). Table 6.1 shows the list of various NPs with their inhibitory mechanism against MDR microbes.

6.3.1 Nitric Oxide-Releasing Nanomaterials

In recent years, gas-releasing agents especially nitric oxide-releasing NMs (NO-NMs) have found applications in combating multidrug resistance in microbes (Rong et al. 2019). NO released from NMs reacts with superoxide ($O_2^{\cdot -}$) to produce reactive nitrogen intermediates (RNOS) causing bacterial cell death. NO (>1 mM) shows toxicity against microbes by various mechanisms (Wang et al. 2017c; Nguyen et al. 2016) including (i) interaction of RNOS with prosthetic groups of proteins, (ii)

inducing nitrosative damage to DNA, (iii) reacting with bacterial protein residues, (iv) lipid peroxidation, and (v) interference with zinc metalloproteins to hinder cellular respiration. Additionally, NO can also trigger the immune response in humans and animals (Dolansky et al. 2018).

Kafshgari et al. reported the antibacterial efficacy of NO-releasing porous silicon NPs (Hasanzadeh Kafshgari et al. 2016). In the presence of ascorbic acid, NO inhibited *E. coli* and *S. aureus* growth within 2 h of exposure. However, after 24 h of exposure, bacterial growth decreased to 1 log than untreated cells. Gehring et al. have also demonstrated the antibacterial activity from NO-releasing mesoporous organosilica (Gehring et al. 2016). The antibacterial effect of NO alone and along with gentamicin in polymeric NMs was also investigated (Nguyen et al. 2016). It was found that both agents were simultaneously released and displayed a synergistic effect causing a decrease in the planktonic cell viability and biofilm formation by 95% and 90%, respectively. NO-NMs are also reported to cause interference in adhesion of MR *S. aureus* and also prevent the biofilm formation when tested in rat central venous catheter model of infection (Mihu et al. 2017). NO-releasing silver NPs (AgNPs) are also reported to show antibacterial activity. The presence of NO on the particle surface enhances the antibacterial effect due to the synergistic effect of AgNPs and NO (Seabra et al. 2017). So far, there is no report on the development of resistance against NO, which could be due to the no increase in minimum inhibitory concentration (MIC) of exposure (Privett et al. 2012). However, some bacteria express enzymes (flavohemoglobin, DNA repair enzymes, lactate dehydrogenase) that protect them from the nitrosative effect of NO at a physiological quantity of NO, but at an adequate concentration of NO, these enzymes also become ineffective (Hall et al. 2020). At a concentration of 1.25–5 mM, NO-NMs are reported to completely eradicate the bacterial cells and also lower the bacterial burden when applied on lesions and intramuscular and dermal abscesses (Schairer et al. 2012).

NO-NMs are also reported to be effective against fungal infections. Bio-screen C analysis and time-lapse microscopy showed NO-NMs induced inhibition of fungal colonies by decreasing the cell division, filament, and bud formation (Rosen et al. 2016). NO-NMs are effective against the *Candida albicans* biofilm formation activity (Hetrick et al. 2009). NO-NMs treatment was found to decrease fungal load and accelerate wound closure in mice. This is also supported by the tissue histology showing a lack of fungal hyphae structures within the dermis and decreased inflammation with increased fibrin and collagen deposition.

6.3.2 Metal-Based Nanoparticles

Different metallic NMs, i.e., gold (Au), silver (Ag), zinc (Zn), magnesium (Mg), and titanium (Ti), prevent drug resistance in microbes by employing a different mechanism to hinder the growth (Wyszogrodzka et al. 2016). Polyclonal antibody-decorated bismuth NPs are reported to enhance the effect of X-ray irradiation to eradicate the MDR bacterial species. The result showed a significant antibacterial effect due to the combined action of bismuth and X-ray, thus killing ~90% of

bacteria, whereas only ~6% death was observed when only X-ray was exposed (Luo et al. 2013). On the other hand, no significant toxicity was observed when human cells were exposed to same concentration. Some of the common metallic NPs that are reported to exhibit antimicrobial effects are discussed below.

6.3.2.1 Titanium Dioxide Nanoparticles

Titanium dioxide NPs (TiO_2 NPs) exert antimicrobial action possibly by the following two mechanisms: (i) production of reactive oxygen species (ROS) when irradiated in the near-UV region leading to the damage of bacterial cell membrane (Ranjan and Ramalingam 2016) and (ii) TiO_2 NPs itself inhibit the growth of microbes by an unknown mechanism (Venkatasubbu et al. 2016). In this context, Liu et al. demonstrated the antibacterial activity of TiO_2 nanocrystals with [101] [001] surface heterojunction promoting electron-hole spatial separation at [101] and [001] facets leading to ROS generation and thus antimicrobial effect (Liu et al. 2017). SEM images revealed that the surface of both *E. coli* and *S. aureus* has been altered by the TiO_2 nanocrystals and the antibacterial effect was due to the depletion of glutathione, membrane lipid peroxidation, and intracellular oxidative stress. Further, Arora et al. reported the antibacterial activity due to the use of a combination of TiO_2 NPs, ceftazidime and cefotaxime in MDR *P. aeruginosa* isolated from sputum, pus, endotracheal tract, and bronchoalveolar lavage (Arora et al. 2015). NPs showed toxicity at 350 μg/mL concentration in presence of UV light for an hour.

6.3.2.2 Zinc Oxide Nanoparticles

Zinc oxide NPs (ZnO NPs) are reported to exhibit antibacterial activity regulated by different mechanisms and also reduce the likelihood of resistance development (Sirelkhatim et al. 2015). Polyvinyl alcohol-coated ZnO NPs showed rapid internalization into the cell cytoplasm by increasing the permeability of the bacterial membrane and inducing oxidative stress within the cytoplasm. In the cytoplasm, ZnO NPs bind to bacterial membrane and destroy the lipid and membrane protein to release the cytoplasmic content, thus resulting in cell death. Additionally, ZnO NPs also produce Zn^{2+} ions, which may rupture the bacterial membrane and promote intracellular ROS generation (Sirelkhatim et al. 2015; Siddiqi et al. 2018). In a study by Patra et al., ciprofloxacin-functionalized ZnO NPs were developed to demonstrate the antibacterial activity against MDR *E. coli*, *S. aureus*, and *Klebsiella* sp. Results showed that this nanoconjugate has a lower MIC than only drug (Patra et al. 2014). Further, the two antibiotics (ciprofloxacin and ceftazidime) were conjugated with ZnO NMs and tested against MDR *Acinetobacter baumannii* (Ghasemi and Jalal 2016). Results showed that there was an increase in the internalization of antibiotics in bacterial cells that supported the change in shape of cells from rods to cocci form. ZnO NPs conjugated with clinically approved drugs (ceftriaxone,

amphotericin B, quercetin, naringin, and ampicillin) have also been evaluated for their antibacterial effect against gram-positive and gram-negative bacteria. Ceftriaxone- and ampicillin-conjugated ZnO-NMs exhibited high antibacterial activity but were found nontoxic to human cells (Akbar et al. 2021).

6.3.2.3 Silver Nanoparticles

Antimicrobial property of AgNMs is mainly considered due to the slow release of silver ions (Ag^+) in an aqueous solution (Ramalingam et al. 2016). Ag^+ react with the bacterial cell membrane and rupture them to release cytoplasmic content leading to cell death. Comparatively, gram-positive bacteria are less sensitive than gram-negative bacteria to Ag^+ exposure, which could be due to the thin cell wall in the latter case (Dakal et al. 2016; Li et al. 2019b). Ag^+ are also reported to be less likely to penetrate the gram-negative bacterial cells due to its strong binding to negatively charged lipopolysaccharide (LPS) of gram-negative bacteria (Acharya et al. 2018). Further, some other mechanisms suggested in the favor of Ag^+ ions exerting antimicrobial effect are (i) damage to genetic material and prevention of p DNA duplication, thus arresting cell division, (ii) binding with cytochrome to interfere electron transport chain, and (iii) inhibition of cell wall formation in gram-positive bacteria (Brown et al. 2012; Munir et al. 2020).

AgNPs are effective against a wide range of pathogens, including drug-resistant fungus, bacteria, and viruses. AgNMs bactericidal effects have been reported against ampicillin-resistant *E. coli* and *S. pyogenes* and MDR *P. aeruginosa*. Combining AgNMs with several drugs (amoxicillin, penicillin G, vancomycin, clindamycin, and erythromycin) is reported to exhibit significant antimicrobial activity (Kaur et al. 2019; Li et al. 2019b). Wang et al. reported the synergistic antibacterial effect of levofloxacin decorated on Ag core-embedded silica nanoplatform (Ag@MSNs@LEVO) against drug-resistant bacteria (Fig. 6.3) (Wang et al. 2016). Results showed that upon treatment with the Ag@MSNs@LEVO to in vivo acute peritonitis model, *E. coli* infection in the peritoneal cavity of the mice reduced to three-fold and pathological effects from spleen and peritoneum were also found to be vanished without exerting any toxic side effect on mice. Thus, this data strongly suggests that Ag@MSNs@LEVO has the potential to be a safe therapeutic option for clinical drug-resistant infections. Mottais et al. synthesized N-heterocyclic carbene-coated silver complexes (Ag-NHCs) featuring a lipid chain and investigated their antibacterial potency (Mottais et al. 2019). It was found that the aqueous formulation of Ag-NHCs showed a better antibacterial effect against some strains of *S. aureus* and *P. aeruginosa*. Additionally, when combined with cationic lipid and DNA, it can also be used to deliver therapeutic genes to infected lungs via aerosolization. Taken together, the data presented herein suggest the use of n-alkyl chain Ag-NHC as a promising alternative to traditional antibiotics in the treatment of respiratory infections and to fight against the rise of MDR bacteria.

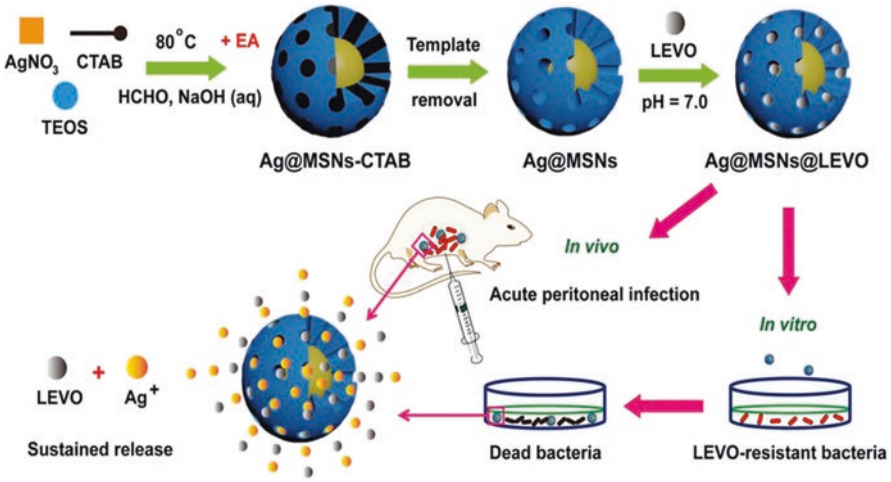

Fig. 6.3 Schematic illustration showing the fabrication of Ag@MSNs@LEVO nanoplatform and its synergistic application over drug-resistant infections in vitro and in vivo. (Reprinted with permission from *Biomaterials,* Copyright 2021, Elsevier (Wang et al. 2016))

6.3.2.4 Copper Oxide Nanoparticles

Copper oxide NPs (CuONPs) are reported as weak but wide spectrum antimicrobial agents, mostly reported effective against *Listeria monocytogenes*, *E. coli*, *S. aureus*, and *S. cerevisiae*. CuONPs utilize two different mechanisms for antimicrobial action: (i) excess amount of Cu ions cause generation of ROS to prevent both DNA replication and amino acid synthesis; and (ii) Cu ions react with amino and carboxyl groups presented on the bacterial surface (Ananth et al. 2015). Agarwal et al. demonstrated the activity of CuONPs against MDR biofilm-forming bacteria (Agarwala et al. 2014). The result showed that CuONPs exposure displayed a zone of inhibition against MR *S. aureus* (22 ± 1 nm) followed by *E. coli* (18 ± 1 nm). It has been reasoned that Cu ions damage the microorganism's envelope and subsequently bind with DNA leading to multiple damages mediated by OH radicals. However, in some cases, it has been reported that copper-mediated oxidative damage follows the Fenton mechanism (Borkow and Gabbay 2009).

6.3.2.5 Bismuth Nanoparticles

Bismuth NPs (BiNPs) are reported to be a potent antimicrobial agent against drug-resistant microbes (Hernandez-Delgadillo et al. 2012). BiNPs are synthesized in different ways with controlled shape and size required to display maximum antibacterial activity (Wang et al. 2008). Besides the use of visible, topical, and UV radiation, X-rays have a high impact to eradicate the bacterial infection when used with BiNPs. It leads to the reduced requirement of radiation dose needed to eradicate

bacteria, thus making it less harmful to humans. Mechanistically, Bi releases electrons by photoelectric effect and generates free radicals following X-ray irradiation, which gradually destroys the bacterial DNA (Luo et al. 2013). Antibiotic conjugation with BiNMs reduces the average distance between targeted microbes and NPs and thus enhances the bactericidal action (Gao et al. 2014). Further, BiNMs radiation therapy is also reported to be highly effective against MDR *P. aeruginosa* (Luo et al. 2013). The bacterial sample was incubated with polyclonal antibody-modified BiNMs and irradiated with X-rays. The result suggested that ~90% of bacteria were killed upon exposure to 200 μg/mL BiNMs, whereas only 6% were killed when exposed to X-ray alone. Additionally, no significant toxicity was observed on human cells, thus establishing the possibility of future clinical applications.

6.3.2.6 Graphene-Based Nanomaterials

Graphene is a single-layer carbon sheet that has been emerging as a potent antimicrobial agent along with other applications. It acts both by physical and chemical methods, and its sharp edges can disrupt the bacterial membrane leading to cell death. Graphene-based NMs (GNMs) are also utilized as dispersing and stabilizing agents for other NMs resulting in high antibacterial competence due to the synergistic effect (Xia et al. 2019). In this context, Aunkor et al. reported the antibacterial activity of graphene oxide (GO) nanosheets against MDR superbugs (*E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *S. marcescens*, and *S. aureus*) obtained from hospitals (Aunkor et al. 2020). Antibacterial activity of GO nanosheets was compared with commonly used antibiotics (azithromycin, cotrimoxazole, ciprofloxacin, amoxicillin, ceftriaxone, imipenem, gentamycin, and cefixime). The result suggested that GO nanosheets may act as "Nano knives" due to the sharp edges and thus rupture the bacterial cell wall. Secondly, bacterial cells may be entrapped in GO nanosheets and detached from the external environment restricting them to access nutrient supply leading to cell death. Entrapment activity depends on the size of nanosheets, and larger-sized nanosheets showed better entrapment (Liu et al. 2012). Further, Wu et al. reported antimicrobial activity of GO in three different MDR bacteria, i.e., *K. pneumoniae*, *E. coli*, and *P. aeruginosa* (Wu et al. 2017). Result demonstrated that upon GO exposure, *K. pneumoniae* colony is eradicated from the agar dish, thus protecting the alveolar macrophage from infection in culture. GO can inhibit the growth and spread of *K. pneumoniae* both in vitro and in vivo which leads to increased cell survival rate, suppressed inflammatory response, less tissue injury, and prolonged mice survival. Further, Pan et al. demonstrated the antibacterial effect of a nanocomposite system based on reduced GO-iron oxide NPs (rGO-IONPs) against MR *S. aureus* (Pan et al. 2016). Mechanism of rGO-IONPs antibacterial effect was suggested due to the heat and OH radical generation causing bacterial cell death both in vitro and in vivo (Fig. 6.4). These findings suggest that GO may be used as promising NMs for efficiently combating MDR infections.

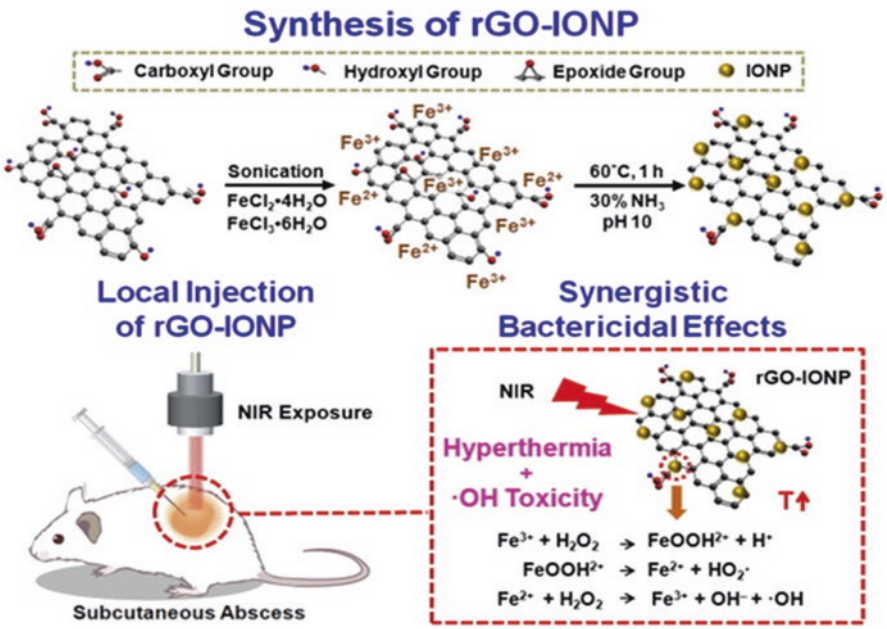

Fig. 6.4 Schematic demonstrating rGO–IONP synthesis and their mechanism of action to inactivate MRSA in subcutaneous abscesses created in a mouse model. (Reprinted with permission from *Nanomedicine: Nanotechnology, Biology and Medicine,* Copyright 2021, Elsevier (Pan et al. 2016))

6.3.2.7 Bimetallic Nanomaterials

Bimetallic NPs composed of AgNPs and AuNPs have been extensively investigated for their antibacterial activity (Singh et al. 2016). AgNPs are a well-known antimicrobial agent. Since the functionalization of AgNPs with biomolecules and drugs remains challenging, therefore, use of bimetallic/alloy NPs has been synthesized to realize its efficient antibacterial property. AuNPs, being biocompatible, are reported as an ideal vector for the delivery of pharmacological compounds. Bimetallic NMs display superior electrical, optical, and catalytic characteristics than their monometallic counterparts (Latif ur et al. 2015). AuNPs and AgNPs bimetallic NMs comprise the properties of both individual NMs, i.e., antimicrobial activity of silver with stability and easy surface functionalization provided by gold (dos Santos et al. 2012). In this context, Fakhri et al. demonstrated the synthesis and functionalization of bimetallic AgAuNPs with tetracycline. The result showed that in combination with bimetallic NPs, antibiotics show a synergistic effect and produce high bactericidal results than their free forms (Fakhri et al. 2017). Recently, Baker et al. synthesized AgAuNPs from the cell-free supernatant of *Pseudomonas veronii* strain AS41G inhabiting *Annona squamosa L.* and demonstrated their antimicrobial efficacy against bacitracin-resistant strain of *B. subtilis*, *E. coli*, and *K. pneumoniae*. Result showed that the synergistic antibacterial effect with antibiotics, bacitracin, kanamycin, gentamicin, streptomycin, erythromycin, and chloramphenicol resulted

in 87.5, 18.5, 11.15, 10, 9.7, and 9.4% fold increase in the activity, respectively (Baker et al. 2017). Further, bimetallic NPs of Au and platinum (AuPtNPs) have also shown enhanced antibacterial activity against sensitive and drug-resistant bacteria (Zhao et al. 2014). Mechanism of action revealed the elevation of adenosine triphosphate (ATP) level and dissipation of bacterial membrane potential.

6.3.2.8 Silica Nanoparticles and Their Derivatives

Silica NPs (SiNPs) offer a variety of functional properties that make them a useful candidate to fight against bacterial infections and are reported to inhibit biofilms from wearable medical implants (Selvarajan et al. 2020). In this context, Kanugala et al. demonstrated the antibacterial activity of phenazine-1-carboxamide (PCN)-loaded SiNPs (PCN-SiNPs) against planktonic *C. albicans* and biofilms of *C. albicans-S. aureus* (Kanugala et al. 2019). Results showed that the antimicrobial activity of PCN-SiNPs was enhanced significantly than PCN and SiNPs alone on silicone urethral catheters. The mechanistic study revealed that released PCN induces ROS production in all microbes, thus resulting in disrupted homeostasis, reduced ergosterol content, altered membrane permeability, and leakage of Na^+, K^+, and Ca^{2+}. Similarly, Wang et al. proposed that silica-gentamycin NPs incorporated in gelatin matrix cross-linked on microarc-oxidized titanium could be used for coating percutaneous implants (Wang et al. 2017b). The antibacterial titanium coating was found to be biocompatible and capable of inhibiting the growth of *S. aureus*. It was also found that the nano-delivery system is biocompatible and thus can be utilized to prevent infection around percutaneous implants.

In various cases, loaded drug amount is insufficient because the physically absorbed drugs in the mesopores suffer from quick release during encapsulation. To overcome this limitation, Kankala et al. developed metal-doped SiNPs where metal embedded in the siliceous frameworks acts as an anchor for drug molecules by establishing the coordination interaction (Kankala et al. 2020). Host-guest interaction among metal and ligands facilitates the high loading capacity compared to the naked SiNPs to allow targeted delivery in the acidic environment at the bacterial infection site. Thus, the synthesized nanocomposite consists of cu-doped SiNPs and holds a pH-responsive coordination interaction with the molecule tetracycline. Further, the nanocomposite was coated with ultrasmall AgNPs. The released Ag^+ can sensitize the resistant strain due to interaction with the membrane and damage the cytoplasmic components, by free radical via Fenton-like reaction. This formulation showed no significant toxicity to mammalian fibroblast cells; therefore, it can be concluded that this trihybrid nanocomposite having a synergistic effect and pH-responsive delivery of antibiotics could play a significant role in combating MDR bacterial species.

Efflux pump system-mediated antibiotic discharge is one of the major causes of MDR in bacteria. To overcome this challenge, novel nanocarriers are designed that can significantly inhibit the growth of MDR bacteria by increasing the retention time of antibiotics (Chen et al. 2018). Based on this, Chen et al. demonstrated the

pH-responsive SiNPs nanocarrier coated with folic acid and calcium phosphate via electrostatic interaction and biomineralization, respectively (Chen et al. 2018). Further, the nanocarrier was loaded with ampicillin with increased uptake and reduced efflux effect in *E. coli* and *S. aureus* via folic acid targeting. The mechanistic study revealed that nanoformulation reduced the protein content and also inhibited the protein activity in MDR bacteria, which leads to the destruction of the bacterial membrane and finally cells' death.

6.3.2.9 Iron Oxide Nanoparticles

Reports suggest that antimicrobial activity of iron oxide NPs (IONPs) is mainly due to ROS generation leading to DNA damage, lipid peroxidation, cellular integrity disruption, and release of metal ions. All these events significantly alter cellular homeostasis and biomolecule coordination (Arias et al. 2018). Antibacterial activity of IONPs has been investigated in bacterial species present in the planktonic free state as well as in biofilms. Reports showed that MIC of IONPs-conjugated amoxicillin nanosystem is approximately three to four times lower than the antibiotic alone when tested in *E. coli* and *S. aureus* (Grumezescu et al. 2014). It reduces the bacterial cell adhesion to polystyrene surface at the initial stage of biofilm formation. In addition, IONPs-chitosan (IONPs-CHT)-streptomycin nanosystem exhibited a significant toxic effect on gram-negative bacteria than gram-positive bacteria (El Zowalaty et al. 2015). Wang et al. demonstrated the IONPs-based silver micro flowers conjugated with antibiotic vancomycin and SiO_2 to act synergistically on MR *S. aureus* and *E. coli* (Wang et al. 2017a). Synthesized nanosystem possesses high magnetic response due to the presence of an iron core of ~200 nm and flower-like Ag shell for offering high surface area and thus release of Ag^+. Vancomycin layer increases the permeability of bacterial membrane by binding to the cell wall, thus allowing the easy entry of Ag^+ to induce cell death. The antimicrobial effect of the nanosystem is reported to be more than 90% effective even after five cycles of assays, which proves the stability of the system. Further, Benjamin et al. have synthesized a biocompatible, multi-compartment nanocarrier consisting of hydrophobic IONPs and hydrophilic methicillin for the treatment of infections associated with medical devices (Fig. 6.5) (Geilich et al. 2017). Applying an external magnetic field, the nanocarrier penetrates ~20 μm thick biofilm of *S. epidermis* and eradicates all bacterial population at 40 μg/mL of IONPs carrying 20 μg/mL methicillin. Most importantly, this formulation was effective against biofilm from MR cells but nontoxic to mammalian cells. Thus, evidence suggests that the growth of antibiotic-resistant biofilms can be overcome by manipulating the arrangement of nanocarriers holding two or more therapeutics. Gabrielyan et al. demonstrated the antibacterial effect of IONPs on ampicillin- and kanamycin-resistant *E. coli* strains (Gabrielyan et al. 2019). The result showed that in the presence of ATPase inhibitor, N,N′-dicyclohexylcarbodiimide, IONPs reduces the H^+ flux through the bacterial membrane by two-fold proving the ATP metabolism-dependent antibacterial activity of IONPs.

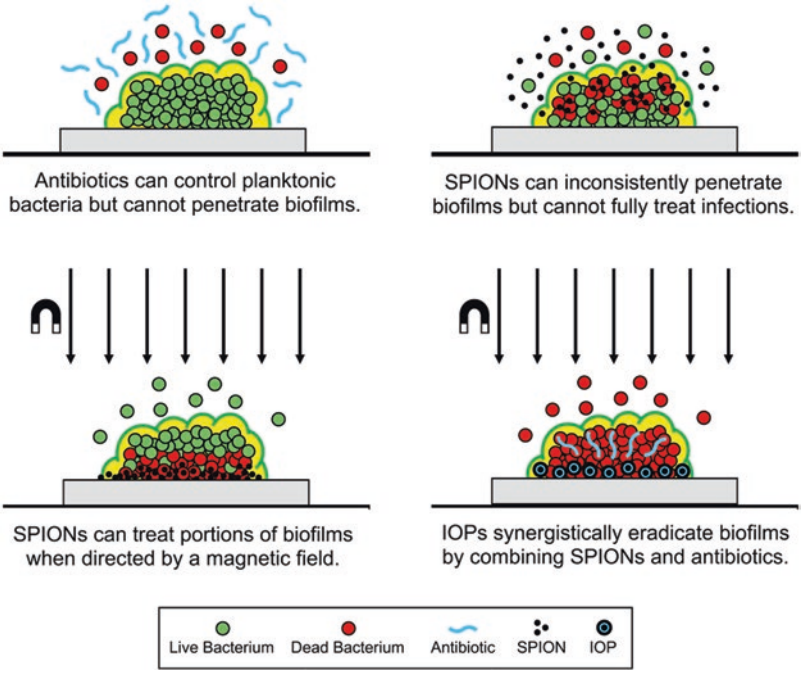

Fig. 6.5 A strategy for biofilm treatment using SPIONs and/or antimicrobials. IOP = iron oxide-encapsulating polymersome. (Reprinted with permission from *Biomaterials,* Copyright 2021, Elsevier (Geilich et al. 2017))

To overcome antibiotic resistance, peroxidase-based therapies have gained tremendous interest because of the knowledge that the peroxidase enzyme is present in blood cells to support innate immunity (Tonoyan et al. 2017). Natural peroxidase enzymes can inhibit microbial infections; however, their application is limited due to the low stability, difficult synthesis and purification process. Nanozymes with peroxidase mimetic activity have shown promising results than natural peroxidase enzymes because the former offers high stability and easy synthesis with tunable properties (Huang et al. 2019). H_2O_2 serves as an initiator in the peroxidase reaction and, therefore, used as a disinfectant in normal practice. However, due to the presence of peroxidase-degrading antioxidant enzymes (SOD and catalase) in bacterial cells, H_2O_2 alone cannot serve the purpose. Thus, a combination of peroxidase mimetic nanozyme with H_2O_2 could be used to kill the bacterial cells by generating $^{\bullet}OH$ radicals (Yin et al. 2016). In this context, Vallabani et al. have demonstrated a synergistic antibacterial mechanism from citrate-coated IONPs in combination with ATP, which facilitates the $^{\bullet}OH$ radical production (Vallabani et al. 2020). The result showed that this strategy exhibits antibacterial activity on both gram-positive and gram-negative bacteria at neutral pH in presence of H_2O_2 and thus can be used as an effective broad-spectrum antibacterial mechanism.

6.3.3 Chitosan-Based Nanomaterials

Chitosan-based NMs (CHT-NMs) exhibit antibacterial effect by various mechanisms including (i) coherence with bacterial and fungal DNA to hinder the transcription and translation processes; (ii) speedy wound recovery as chitosan deposits more collagen III and fibroblasts in addition to inhibition of inflammatory cytokine release; and (iii) removal of the acetyl group from chitosan that causes protonation at pH <6.5 and acquiring a positive charge. Thus, antimicrobial action is due to the osmotic damage caused due to interaction between positively charged molecules with negatively charged microbial cell walls (Ma et al. 2017; Wassel and Khattab 2017).

Encapsulation of chitosan in NMs improves the solubility of chitosan as well as enhanced their antimicrobial activity (Wassel and Khattab 2017). Chitosan promotes surface positive charge and thus facilitates strong interaction between microbes and CHT-NMs. Comparatively, NMs encapsulated in chitosan proved to be more efficient against *E. coli* and *S. aureus* than when used individually. High and low molecular mass chitosan are reported to be more effective against gram-positive and gram-negative bacteria, respectively. In this context, Marangon et al. demonstrated that the combination of chitosan and rhamnolipids (CHT-RL NMs) exhibits enhanced antimicrobial activity against *S. aureus* (Marangon et al. 2020). Rhamnolipids reduce the size and polydispersity index of CHT-NMs and enhance the surface positive charge to improve stability. CHT-NMs alone can only eliminate the bacteria present in the upper layer of biofilm, whereas CHT-RL NMs are more effective against the sessile bacteria and reduce the viable bacterial cells below the detection limit. This may be attributed to increased delivery of chitosan and rhamnolipid to the bacterial cell surface and consequently to their targets in gram-positive bacteria.

6.3.4 Aptamer-Conjugated Nanoparticles

The application of aptamer-conjugated NMs is also proved to be an attractive strategy to significantly enhance the efficiency as a novel class of antibiotics (Gao et al. 2018; Gutiérrez-Santana et al. 2020). Friedman et al. have demonstrated that highly stable 2-fully modified RNA aptamers could be used for targeted delivery of biomaterials. Modification of anti-SpA (*S. aureus* Protein A) aptamer with fGmH (2-F-dG, 2-OMe-dA/dC/dU) provides resistance to aptamers against alkaline hydrolysis and nucleases present in serum. Further, this aptamer was conjugated with AgNPs to show SpA-dependent antimicrobial effect (Friedman et al. 2015). Moreover, the antimicrobial effect of NMs can be further enhanced by conjugation with different aptamers to target the same pathogen. Song et al. demonstrated the TiO_2 NPs conjugated with aptamers specific for *E. coli* surface-specific ssDNA (three different aptamers). Results showed that the TiO_2 conjugated with three different aptamers

eradicated 99.9% of bacteria in 30 min than TiO_2 attached to a single aptamer (60 min) (Song et al. 2016). Due to the proximity between the aptamer and *E. coli*, there is an efficient and fast ROS transfer to the cells causing cell death. The developed nanosystem was found to be specific to *E. coli* even from the mixed culture of *E. coli* and *S. epidermidis*.

Single-wall carbon nanotubes (SWNTs) have been explored for antimicrobial activity. Using a selective aptamer conjugated to ciprofloxacin (Apt-CPX) and SWNTs, ~90% of *P. aeruginosa* biofilm inhibition was achieved (Apt-SWNTs) (Wang et al. 2018). Further, Yeom et al. demonstrated AuNPs-conjugated aptamer for delivery of antimicrobial peptide (AMP) to mammalian cells (Yeom et al. 2016). The result showed that treatment of nanoformulation increased the viability of host cells as well as inhibited the colonization of bacteria and thus promoted a 100% survival rate of infected mice. Therefore, the conjugate served as an efficient and novel agent to eradicate intracellular bacterial infection. Immobilization of vancomycin in the pores of SiNPs and their subsequent conjugation with an anti-*S. aureus* aptamer are an effective strategy for antibiotic delivery to the targeted site and also reduce the MIC and toxicity of pure antibiotics against other related species.

6.4 Use of Nanomaterials in Combating Bacterial Diseases of Animals

Bacterial diseases in animals of industrial importance are becoming a serious global concern. The common animal bacterial diseases are brucellosis (*Brucella melitensis*, *Brucella abortus*, *Brucella canis*), septicemia (*Pasteurella multicoda* and *E. coli*), mastitis (*Staphylococcus aureus*, *Staphylococcus dysgalactiae*, *Staphylococcus agalactiae*), listeriosis (*Listeria monocytogenes*), salmonellosis (*Salmonella enterica*), bovine tuberculosis (*Mycobacterium bovis*), anthrax (*Bacillus anthracis*), etc. Pathogens of these disease are transmitted from animals to humans and cause various disorders (Fig. 6.6). Although there have been several strategies adopted to circumvent the onset of these animal diseases, the use of NMs has shown potential in effective prevention. The following section will comprehend only a few important diseases and provide a summary of the progress of various treatment strategies.

6.4.1 Brucellosis

Brucellosis is a bacterial disease caused by various *Brucella* species such as *Brucella melitensis*, *Brucella abortus*, *Brucella canis*, *Brucella neotomae*, *Brucella ovis*, and *Brucella suis*. These species mainly infect goats, cattle, swine/pig, sheep, etc. *Brucella* can also be transmitted to human upon direct contact with infected animal

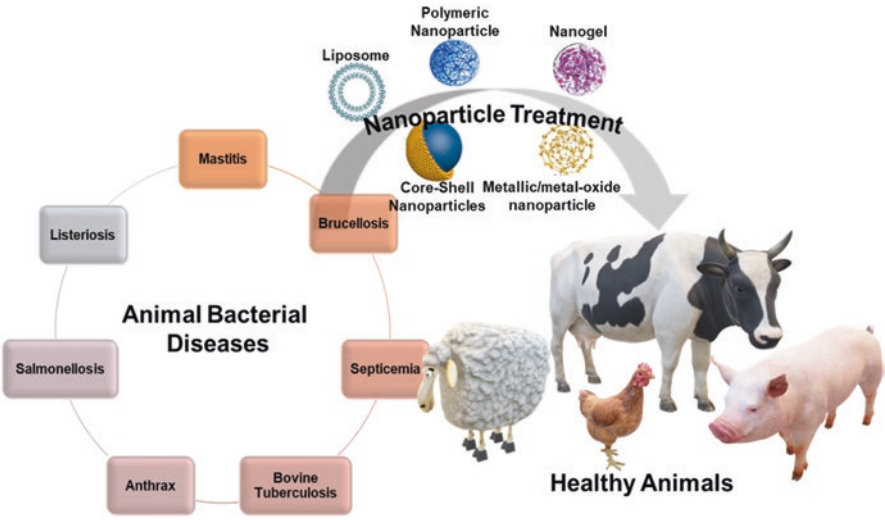

Fig. 6.6 Schematic showing a list of various diseases caused by bacteria in livestock and use of different nanomaterials for the treatment

or due to consumption of contaminated animal products such as unpasteurized milk, raw meat, and other byproducts (Thirumalaivasan et al. 2019). In 2018, a large study from 23 states of India revealed ~12% of cases of brucellosis in cattle and buffalo (Deka et al. 2018) causing an economic loss of ~58.8 million USD per year in the dairy sector (Deka et al. 2018). Brucellosis leads to the reduced milk production, death of young ones, abortion, retained placenta, stillbirths, increased calving intervals, etc. (Holt et al. 2021). Conventional antibiotics are prevalent to be used for the treatment of brucellosis including aminoglycosides, tetracycline, rifampicin, quinolones, doxycycline, streptomycin, and chloramphenicol (Khan, Zahoor 2018). The antibiotics are also delivered as encapsulated in NPs for the treatment of these pathogens. Lueth et al. (Lueth et al. 2019) developed polyanhydride NPs encapsulating doxycycline and rifampicin (individually and in combination) and tested their activity against *Brucella melitensis.* A 1:1 ratio combination of doxycycline and rifampicin in NPs showed the best release performance of drugs under in vivo system (BALB/C mice infected with *Brucella melitensis*). Within 5 days of treatment, the bacterial burden was decreased by 3 $\log_{10}$ times in the liver of mice. A treatment of 21 days led to the bacterial burden in the spleen and liver equal to free drugs (3.5 mg) and nanoformulation (1.5 mg). Thus, the NPs-based delivery improved the drug/s release time and dose sparing without compromising the activity under in vivo system. More examples of the use of NPs to display anti-brucellosis activity are summarized in Table 6.2.

Table 6.1 Nanomaterials frequently used in the treatment of MDR microbes

Nanomaterial type	Size (nm)	Antibiotics used	Targeted microbes	Important observation	References
Conventional liposome (egg yolk lecithin)	75–92.14	Cinnamaldehyde	*S. aureus*	Liposomes improve the stability and durability of cinnamaldehyde and antibacterial efficacy against *S. aureus*	Chen et al. (2019b)
Gold nanoflower	–	Ciprofloxacin	*B. subtilis*, *S. aureus*, *P. aeruginosa*, *and E. coli*	Nanoflower-drug conjugate interacts with the phosphate/amine group present on outer membrane of gram-negative bacteria and exerts its antibacterial activity	Sreedharan and Singh (2019)
Shell-cross-linked knedel-like NPs, AgNPs	22	Minocycline	MDR *P. aeruginosa* and *S. aureus*	Demonstrated enhanced antibacterial effect in comparison to free silver and minocycline at same concentration	Chen et al. (2019a)
Poly lactic-co-glycolic acid (PLGA)	226.00 ± 5.57	Teicoplanin	MR *S. aureus*	~32-fold reduction was observed in the MIC values of *S. aureus* strains when treated with teicoplanin-aptamer-PLGA NPs	Ucak et al. (2020)
Carboxymethyl tamarind polysaccharide capped AgNPs	~20–40	–	MDR *E. coli*	AgNPs inhibit biofilm formation and alter the expression and positioning of bacterial cytoskeletal proteins	Sanyasi et al. (2016)
Vanadium oxide nanodots	3.36 ± 0.23	–	*E. coli* and *S. aureus*	High antibacterial efficiency and good biocompatibility	Ma et al. (2020)
AuRh and AuRu NPs	~5	–	Polymyxin-resistant *E. coli*, *P. aeruginosa*, and *K. pneumoniae*	Destroy cell membrane and increase level of ATP and ROS. AuRh accelerate wound healing caused due to MDR bacteria	Zhao et al. (2020)
CHT-NPs	193 ± 1.9 to 530 ± 13	–	MDR *Neisseria gonorrhoeae*	CHT NPs found to be effective against all tested strains, including multiple antibiotics-resistant strain	Alqahtani et al. (2020)

(continued)

Table 6.1 (continued)

Nanomaterial type	Size (nm)	Antibiotics used	Targeted microbes	Important observation	References
AgNPs	15–20	Amoxicillin, azithromycin, clarithromycin, or linezolid	MR *S. aureus*	Proved to be promising therapy against infections caused due to MR *S. aureus*	Akram et al. (2016)
Oleic acid (OA)-monomethoxy polyethylene glycol (mPEG) nanocarrier	142.9	Vancomycin	MR *S. aureus*	In vivo therapy revealed 1.47-fold greater reduction in bacterial load after treatment with vancomycin-loaded mPEG-OA polymersomes in comparison to bare vancomycin	Omolo et al. (2017)
pH-responsive CHT NPs with new anionic gemini surfactant (AGS)	220.57 ± 5.9	Vancomycin	MR *S. aureus*	At acidic and normal physiological conditions, sustained drug release was reported with enhanced in vivo activity	Kalhapure et al. (2017)
pH-responsive nanostructured lipid carriers (NLC)	225.2 ± 9.1	Vancomycin	MR *S. aureus*	Vancomycin loaded-NLCs had enhanced antibacterial activity and reduced bacterial population 2.5-fold more than the bare drug	Osman et al. (2019)
AgNPs	13.47 ± 12	Alpha-amylase	MDR *K. pneumoniae* and MR *S. aureus*	Demonstrated higher antibacterial activity than AgNPs and α-amylase alone, with significant reduction in the biofilm formation	Abeleda et al. (2020)
CHT-NMs	180 ± 5	Mannose	Resistant *E. coli* and *L. monocytogenes*	Demonstrated enhanced antibacterial activity against biofilm. Inhibit the exponential growth of bacteria by interacting with bacterial membrane	Ejaz et al. (2020)
Graphene/chitosan nanocomposite	–	–	*K. pneumoniae* and *MDR P. aeruginosa*	Inhibited the biofilm-forming strains of *P. aeruginosa* and *K. pneumonia* via membrane damage and disruption of cellular morphology	Muthuchamy et al. (2020)

6.4.2 Septicemia

Septicemia is a type of blood poisoning caused by bacteria through the release of toxins leading to death in livestock. Hemorrhagic septicemia and colisepticemia mainly affect cattle, chickens, and water buffalo. Recovery from this disease is very rare. Hemorrhagic septicemia and colisepticemia are caused by *Pasteurella multicoda* and *E. coli.* According to a study, ~ 792 million USD per year economic loss in India is caused by hemorrhagic septicemia (Singh et al. 2014). Symptoms could be depression, fever, decreased milk production, nasal discharge, swelling on the neck and brisket, dyspnea, abdominal pain, and meningitis. A combination of streptomycin and penicillin, oxytetracycline, trimethoprim, ampicillin, doxycycline, and other antibiotics has shown promising results if the treatment is given at the early stage of the disease (Liu et al. 2018; Zhang et al. 2017). Vaccines could protect animals for 6–12 months. The vaccine is developed using a virulent *Pasteurella multicoda* strain B:3–4, dense bacterin combined with oil or alum as an adjuvant, and formalin-inactivated bacterin (Li et al. 2019a). Awaad et al. (Awaad et al. 2021) studied the clinical and pathological performance of AgNPs on colisepticemia in broiler chickens infected by the exposure of 3×10^8 CFU/mL of *E. coli* (serogroup O78) for 2 days. Chickens fed on 4, 6, and 8 mg/kg of AgNPs revealed that 4 mg/kg dose significantly reduced the bacterial burden, histopathological lesion scores, and virulence of genes. A dose of AgNPs (8 mg/kg) resulted in a severe negative effect on chicken health.

6.4.3 Mastitis

Mastitis is a fatal infection of the mammary gland in cattle, which occurs when bacteria enter a milk duct via a crack in a nipple. Different species of bacteria such as *S. aureus*, *Staphylococcus dysgalactiae*, *Staphylococcus agalactiae*, coagulase-negative *Staphylococcus, Staphylococcus uberis, Enterococci*, coliform bacteria *E. coli*, etc. cause mastitis. The symptoms include reduction in milk production, poor milk quality, swelling and redness in the udder, pus, or clotting in milk (Yashchenok et al. 2012). In India, mastitis is estimated to decrease milk production by 21%, costing about Rs. 575 million USD per annum (Sharun et al. 2021). Common antibiotics such as ampicillin, cloxacillin, tetracycline, penicillin, and streptomycin are used as an ointment and intravenous or intramuscular injection for the treatment of mastitis (Yang et al. 2019). Among NPs-based treatment, Cardozo et al. (Cardozo et al. 2014) synthesized polymeric NPs releasing NO radicals against MDR *S. aureus* and *E. coli.* The polymeric NPs encapsulating mercaptosuccinic acid (MSA) (S-nitroso-MSA particles) worked well as a NO donor. This formulation showed a sustained formation of NO radicals in presence of MDR bacterial strains (MIC 125–250 μg/mL).

6.4.4 Listeriosis

Listeriosis is a food-borne intracellular infectious disease of animals, birds, fishes, and humans. Causative bacteria (*Listeria monocytogenes*) are present in soil, plants, mud, and stream. Goats, sheep, and cattle get sick from eating contaminated corn silage. In India, ~ 7.66% of animals were found to have listeriosis (a survey conducted from 2015 to 2018) (Chaudhari et al. 2021). Common symptoms of listeriosis are inflammation in the brain, loss of balance, dystonia, loss of appetite, fever, etc. Penicillin, sulfonamides, ampicillin, tetracycline, etc. are traditional antibiotics used for the treatment (Dhama et al. 2015). Mohammed and Abdel Aziz (2019) evaluated the biocidal activity of AgNPs alone and in combination with different commercial disinfectants (sodium hypochlorite, H_2O_2, Virkon®S, benzalkonium chloride, and ammonium compound TH^{4+}®) on MDR species of *L. monocytogenes* isolated from 260 samples of animal and human stool. Among the different combination, 2% Virkon®S/AgNPs showed highest antibacterial activity (100%) followed by 5% H_2O_2/AgNPs (90%) and 1% TH^{4+}/AgNPs (90%).

6.4.5 Salmonellosis

Salmonella (*Salmonella enterica*), a gram-negative, rod-shaped bacterium, causes salmonellosis in warm-blooded animals. *Salmonella* is commonly found in contaminated food, the stool of animals, and the intestine of various animals. Different species of *Salmonella* infect different animals causing typhoid-like symptoms such as *Salmonella Gallinarum* in poultry, *Salmonella Abortusovis* in sheep, *Salmonella Choleraesuis* in pigs, *Salmonella Dublin*, and *Salmonella Typhimurium* in cattle. Diarrhea, fever, and abdominal cramps are the major symptoms of salmonellosis (Duraisamy 2016). For the treatment of salmonellosis, a high concentration of antibiotics is found to be effective when delivered at the infected intracellular site (small intestine). Among different NPs-based formulations, Xie et al. (2017) have developed enrofloxacin-encapsulated solid lipid NPs (SLN) for the effective intracellular delivery of the drug. Enrofloxacin-loaded SLN (0.24 and 0.06 μg/mL) showed ~99.97% inhibition in *salmonella CVCC541* (3.80 CFU/mL) growth, whereas same inhibition could be achieved by 0.6 μg/mL of free enrofloxacin (4.15 CFU/mL).

6.4.6 Bovine Tuberculosis

Mycobacterium bovis causes chronic disease, bovine tuberculosis, in pigs, goats, cattle, deer, cats, and dogs. Contaminated food and water are some of the common sources of infection. Bovine tuberculosis is commonly found to affect the lymph glands of the throat and lungs of infected animals. In 2017, 7.3% of the Indian (300

million cows and buffalos) population was found to be infected with bovine tuberculosis (Mydin et al. 2018) causing a ~4% decrease in overall milk production (Rinu et al. 2020). In cattle, mostly respiration-related symptoms (coughing, lymph node enlargement, dyspnea) are realized without any significant clinical signs (Lee et al. 2001). Rifampicin, isoniazid, pyrazinamide, kanamycin, ethambutol, etc. are the traditional antibacterial drugs used for the treatment of *Mycobacterium bovis* infection (Marianelli et al. 2015). Small cationic peptides as antimicrobials are recently reported as one of the best alternatives for the treatment of bovine tuberculosis (AlMatar et al. 2018). Zhou and coworkers (Liang et al. 2020) used PLGA NPs encapsulating small recombinant bovine neutrophil β-defensin-5 (PLGA-B5 NPs) as an antibacterial agent. In in vitro studies (on J774A.1 cells), PLGA-B5 NPs enhanced the expression of IL-1β, tumor necrosis factor, and IL-10. After 4 weeks of PLGA-B5 NPs treatment, *Mycobacterium bovis*-infected BALB/C mice showed a significant decrease in bacterial burden in the lungs, pulmonary area, and spleen. More examples of the use of NPs to display anti-bacterial activity is mentioned in Table 6.2.

6.4.7 Anthrax

Anthrax is a bacterial disease caused by spore-forming *Bacillus anthracis*, commonly infecting livestock animals. According to the study, ~28% of cattle are infected with *Bacillus anthracis* globally (Sushma et al. 2021). The major symptoms of the infection include abrupt fever, convulsions, staggering, depression, cardiac distress, etc. Various antibiotics are reported for the treatment of anthrax such as ofloxacin, gentamicin, doxycycline, imipenem, etc. (Weiss et al. 2011). Sun et al. (2016) synthesized a visible light-responsive carbon-containing TiO_2 NPs (C-TiO_2-NPs), causing a significant increase (~60%) in antibacterial activity than TiO_2 NPs alone. Further, C-TiO_2-NPs with visible light treatment cleared ~90% of anthrax lethal toxins (major virulence factor for anthrax). In another study by Manayani et al. (2007), chimeric virus-like NPs (CV-NPs) were synthesized to work as an antitoxin against an AB-type toxin generated by *Bacillus anthracis*. CV-NPs generated a complex system with protective antigens, which further facilitated the clearance of the toxins and generation of immune response in Sprague-Dawley rats.

6.5 Nanoparticle-Based Antibacterial Strategies in Clinical Studies

A number of nanosystem-based antibacterial strategies are being evaluated in clinical trials (Eleraky et al. 2020). Few strategies are being discussed in the following section.

Table 6.2 Application of NPs for the treatment of animal diseases caused by bacteria

Nanoparticle	Bacterial disease	Bacterial species	Study model	Observations/results	References
Gentamicin-loaded polymeric NPs (GP-NPs)	Brucellosis	*Brucella melitensis*	In vitro: THP-1 macrophage cell line In vivo: BALB/C mice infected with *B. melitensis*	GP-NPs, delivered to liver and spleen, reduced the bacterial infection in THP-1 cell line by 2-$\log_{10}$. Four doses (18 mg/L) of GP-NPs reduced ~50% infection without any adverse effect	Imbuluzqueta et al. (2013)
Curcumin-loaded niosome hydrogel-doxycycline-loaded chitosan-sodium alginate NPs	Brucellosis	*Brucella melitensis*, *Brucella abortus 544*, and *Brucella suis 1330*	Guinea pigs infected with different *Brucella* species	The formulation showed sustained release of curcumin and doxycycline but did not completely remove the artificially created *Brucella* infection. However, bacterial load was significantly lowered (~90%) from spleen and blood	Abo El-Ela et al. (2020)
Cerium oxide NPs (CeNPs)	Septicemia	*E. coli* and *S. aureus*	Sprague-Dawley rats injected with cecal materials to induce sepsis	Exposure of CeNPs to *E. coli* and *S. aureus* led to inhibition of growth of bacteria due to the reduced ROS level and decrease in NF-kB/p65 binding to DNA in rats. Lower levels of IL-6 and blood urea nitrogen were observed suggesting reduced inflammation	Selvaraj et al. (2015)

Melatonin-encapsulated polymeric (poly(ethylene glycol) (PEG) and poly(propylene sulfide) (PPS)) NPs (PEG-PPS-NPs)	Septicemia	*E. coli* serotype O127:B8	C57BL/6 J mice	The formulation released melatonin on demand (ROS-mediated delivery) in in vivo system with highest concentration in the liver, kidney, lung, and pancreas than control groups. This indicates that ROS-mediated delivery of melatonin from the PEG-PPS-NPs works against septicemia	Chen et al. (2017)
2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO)-coated polymeric NPs (TEMPO-PNPs)	Listeriosis	*Listeria monocytogenes*	BALB/C mice infected with *Listeria monocytogenes*	TEMPO-PNPs alone did not show significant reduction in bacterial count; however, they protected organs from the oxidative damage due to the infection. A combination of amoxicillin with TEMPO-PNPs resulted in decreased bacterial counts in infected mice	Ikeda et al. (2018)
AgNPs	Listeriosis	*Listeria monocytogenes*	BALB/C mice infected with *Listeria monocytogenes*	AgNPs treatment reduced the bacterial count (10 CFU/mL) in the small intestinal and decreased the oxidative stress and inhibited expression of pro-inflammatory cytokine genes	El-Zamkan et al. (2021)

(continued)

Table 6.2 (continued)

Nanoparticle	Bacterial disease	Bacterial species	Study model	Observations/results	References
AgNPs	Mastitis	*S. aureus* and *Pseudomonas aeruginosa*	Bacterial samples from milk were collected from mastitis-infected goats	AgNPs-treated bacteria showed decreased lactate dehydrogenase activity and adenosine triphosphate levels than control group. Expression of glutathione, SOD, and catalase enzymes was downregulated, but glutathione S-transferase expression was promoted	Yuan et al. (2017)
AgNPs and copper NPs (CuNPs), Ag-Cu complex	Bovine mastitis	*Staphylococcus agalactiae*, *Staphylococcus dysgalactiae*, *Enterococcus faecalis*, *S. aureus*, *Salmonella enteritidis*, *E. coli*, and *Enterobacter cloacae*	–	AgNPs (200 ppm), CuNPs (50 to 100 ppm), and Ag-Cu complex (200 ppm) showed inhibition in biofilm formation by different bacterial species	Lange et al. (2021)
Chitosan-adjuvanted *Salmonella* subunit NPs (CSNPs)	Salmonellosis	*Salmonella*	White Leghorn layer chickens	The mucoadhesive oral drinking water containing CSNPs was used as a nanovaccine that increased the expression of different Toll-like receptors and Th1 and Th2 cytokines in chicken immune cells	Renu et al. (2020)
ZnO NPs	Salmonellosis	*Salmonella typhimurium* and *S. aureus*	–	Results revealed that ZnO NPs (1.33 mM) ruptured the cell wall of the bacteria which resulted in cell death	Akbar et al. (2019)

TiO_2 NPs	Bovine tuberculosis	*Mycobacterium bovis*	–	TiO_2 NPs (10–100 μg/mL) inhibited the biofilm formation. The metabolic activity of *Mycobacterium bovis* was decreased by threefold. Also, this particle was biocompatible to normal lung bronchus cells up to 100 μg/mL concentration	Ramalingam et al. (2019)
PLGA NPs encapsulated with argF antigen	Bovine tuberculosis	*Mycobacterium bovis*	Specific pathogen-free BALB/C female mice	NPs-treated BALB/C mice showed enhanced response of IgA, interferon-λ, and CD^{4+} T cells against *Mycobacterium bovis* infection. Also, inflammatory lesions in lung tissue and bacterial burden were found to be decreased after treatment	Ni et al. (2021)

6.5.1 Nanoparticles Delivering Antibiotics

First clinical use of ciprofloxacin-loaded liposome known as Lipoquin was under phase 1 trial in some healthy volunteers (Bruinenberg et al. 2010), and then it entered in phase 2 trial of 14 days, on 21 adults, to evaluate the initial safety, activity, and pharmacokinetics (one-time inhalation every day). Simultaneously, a double-blind and randomized phase 3 trial (ORBIT-3 and ORBIT-4) was conducted internationally in a similar region to investigate the safety and efficacy of Lipoquin (Haworth et al. 2019). Further, amikacin-loaded liposomes were also studied in clinical trials. In a double-blind phase 2 trial, efficacy, tolerability, and safety of once-daily (QD) dosing of amikacin (590 mg) versus placebo was conducted for 84 days against refractory *nontuberculous mycobacteria* lung infection. Another study also examined the stability and safety of once-daily dosing of 560 mg amikacin-loaded liposome for six cycles over 18 months in the patient suffering from cystic fibrosis and chronic infection by *P. aeruginosa* (Eleraky et al. 2020).

A new study of phase 2 trial of inhaled liposomal amikacin 590 mg, once-daily dosing for 12 months has been evaluated for their safety, tolerability, and efficacy of treatment against *Mycobacterium abscessus* lung disease. Further, a phase 3 trial was also conducted to study the safety and long-term tolerability of inhaled amikacin-loaded liposome (590 mg/day) in the patient suffering from chronic infection of *P. aeruginosa* (Eleraky et al. 2020).

6.5.2 Nanoparticle Delivering Antimicrobial Peptides and Antitoxins

Antimicrobial peptides exhibit less chance of resistance development and produce wide-spectrum antibacterial activity (Molchanova et al. 2017). They mainly target bacterial cell membrane synthesis of protein, nucleic acid, cell wall, enzymatic activity, and ATP efflux process. Some of the antimicrobial peptides are nisin (interfere in cell wall synthesis); indolicidin (interfere in protein synthesis); buforin II (inhibit RNA synthesis); and histatins (alter ATP efflux) (Molchanova et al. 2017). In the treatment of bacterial-driven diseases, targeting the bacterial components which are responsible for their virulence, i.e., toxins, is a major aspect of nanomedicine these days. Bezlotoxumab is a human monoclonal antibody and the first approved antitoxin in 2016 which is designed to target the toxin B of *Clostridium difficile* (Mullard 2016). Several antitoxin agents are still in clinical trials like monoclonal antibodies targeting *S. aureus*' α-toxin and type III toxin secretion moiety of *P. aeruginosa* (Azeredo da Silveira and Perez 2017). Further, a novel empty liposome, CAL02, has been developed which leads to a synergistic effect with drugs and antibiotics and also demonstrated the ability to rescue mice from major infections, such as staphylococci, through the adsorption of toxins (Laterre et al. 2019).

6.5.3 *Limitations of Nanoparticle-Based Antibacterial Agents*

Clinical translation of NPs-based antibacterial agents faces several challenges including biocompatibility, safety, laws and regulations, intellectual property rights (IPRs), and high cost than traditional therapies (Narang et al. 2013; Hua et al. 2018). Important issues which should be considered during clinical translation of nanomedicine are:

(i) *Preclinical evaluation of toxicity*: To avoid the side effects of nanomedicine, they should be initially evaluated in vivo (inappropriate animal models) and analyze their pharmacokinetics and pharmacodynamics properties. Further, biocompatibility and stability of NPs and interaction with the surrounding medium should also be considered (Eleraky et al. 2020).

(ii) *Nanopharmaceuticals design*: In the designing of antimicrobial nanoformulations, factors like biodegradability, administration route, and physical and chemical stability should be considered. Large-scale production of antimicrobial drugs should consider the factors like reproducibility and quality control assay, i.e., polydispersity, the storage stability of final product, charge, morphology, incomplete purification, and consistency of nanomedicine (Tinkle et al. 2014; Teli et al. 2010).

(iii) *Challenges in commercialization*: Launching antimicrobial products in the market is a complex process as it is time- and cost-intensive. Simple techniques should be employed to test the therapeutic efficacy of antimicrobial drugs in the patient. With the use of NMs, biological half-lives of drugs have been greatly enhanced; thus, specialized toxicological tests in animals should be conducted to examine both short- and long-term side effects of antimicrobial nanomedicine (Eleraky et al. 2020). Thus, regulatory guidelines should be developed to examine the nanotoxicological effect and for standard and validated use of NPs in clinical development (Accomasso et al. 2018).

6.6 Conclusion and Expected Future Developments

With the increasing incidence of resistance against antibiotics, it is essential to find alternative methods exhibiting strong antimicrobial activity. In this quest, NPs-based formulations containing essential oils, antimicrobial peptides, and other natural products have been explored. Several studies have confirmed that these NPs-based formulations of certain NPs themselves are better in inhibiting the growth of pathogenic bacterial species than their non-formulated counterparts. Although there are several vaccines available to protect humans from bacterial pathogens, the emergence of new bacterial epidemics and pandemics would require significant efforts for the quick development of new vaccines. Traditional methods of vaccine development take several years; therefore, novel methods must be explored to reduce the development phase in months. Nanotechnology could play an important role to act

as adjuvants to facilitate the delivery of vaccine components. Currently, multifunctional nanocarriers are developed that could be used to deliver multiple vaccines in one shot. These nanocarriers could either encapsulate or carry the vaccine components on their surface and offer high payload delivery to facilitate quick and long-term immune responses.

There has been a lot of effort devoted to develop nanotechnology-based antibacterial agents and vaccines for bacterial diseases in humans; however, limited efforts are made to protect animals from animal diseases caused by microbes. Vaccines for animal diseases (brucella, anthrax, and foot and mouth disease) are developed; however, they have not been improved for decades. These vaccines face several challenges of storage condition, being ineffective in immunocompromised animals, and requiring multiple doses. In the coming years, nanotechnology-based vaccines could be developed to offer long-term stability and better immune response with one-shot treatment. Oral and intranasal vaccines involving the controlled release of vaccine components would be another area of interest. The NPs-based antibacterial agents for animal diseases are so far limited to certain toxic particles such as ZnO and AgNPs, leaving a huge scope of research in developing novel NMs and nanoformulations to combat the drug-resistant bacterial species. NPs conjugated aptamers or another specific biomolecules-based targeting of particular bacterial species would be required to develop. Novel theranostics would also be required for simultaneous detection and treatment of pathogenic and drug-resistant strains of bacteria. Overall, there remains a tremendous amount of research work for developing novel NPs-based antibacterial materials to effectively and selectively cause damage to pathogenic strains barring non-pathogenic strains, humans, and the environment.

Acknowledgments R. Singh thanks the Natural Science Foundation of Shandong Province (Grant No. ZR2020QC061) and Liaocheng University [318051901], PR China. S. Bhagat acknowledges the Indian Council of Medical Research, India, for providing Senior Research Fellowship.

References

Abeleda HEP, Javier AP, Murillo AQM, Baculi RQ (2020) Alpha-amylase conjugated biogenic silver nanoparticles as innovative strategy against biofilm-forming multidrug resistant bacteria. Biocatal Agric Biotechnol 29:101784. https://doi.org/10.1016/j.bcab.2020.101784

Abo El-Ela FI, Hussein KH, El-Banna HA, Gamal A, Rouby S, Menshawy AMS et al (2020) Treatment of brucellosis in guinea pigs via a combination of engineered novel pH-responsive curcumin niosome hydrogel and doxycycline-loaded chitosan–sodium alginate nanoparticles: an in vitro and in vivo study. AAPS PharmSciTech 21(8):326. https://doi.org/10.1208/s12249-020-01833-7

Accomasso L, Cristallini C, Giachino C (2018) Risk assessment and risk minimization in nanomedicine: a need for predictive, alternative, and 3Rs strategies. Front Pharmacol 9(228). https://doi.org/10.3389/fphar.2018.00228

Acharya D, Singha KM, Pandey P, Mohanta B, Rajkumari J, Singha LP (2018) Shape dependent physical mutilation and lethal effects of silver nanoparticles on bacteria. Sci Rep 8(1):201. https://doi.org/10.1038/s41598-017-18590-6

Agarwala M, Choudhury B, Yadav RN (2014) Comparative study of antibiofilm activity of copper oxide and iron oxide nanoparticles against multidrug resistant biofilm forming uropathogens. Indian J Microbiol 54(3):365–368. https://doi.org/10.1007/s12088-014-0462-z

Akbar A, Sadiq MB, Ali I, Muhammad N, Rehman Z, Khan MN et al (2019) Synthesis and antimicrobial activity of zinc oxide nanoparticles against foodborne pathogens Salmonella typhimurium and Staphylococcus aureus. Biocatal Agric Biotechnol 17:36–42. https://doi.org/10.1016/j.bcab.2018.11.005

Akbar N, Aslam Z, Siddiqui R, Shah MR, Khan NA (2021) Zinc oxide nanoparticles conjugated with clinically-approved medicines as potential antibacterial molecules. AMB Express 11(1):104. https://doi.org/10.1186/s13568-021-01261-1

Akram FE, El-Tayeb T, Abou-Aisha K, El-Azizi M (2016) A combination of silver nanoparticles and visible blue light enhances the antibacterial efficacy of ineffective antibiotics against methicillin-resistant Staphylococcus aureus (MRSA). Ann Clin Microbiol Antimicrob 15(1):48. https://doi.org/10.1186/s12941-016-0164-y

AlMatar M, Makky EA, Yakıcı G, Var I, Kayar B, Köksal F (2018) Antimicrobial peptides as an alternative to anti-tuberculosis drugs. Pharmacol Res 128:288–305. https://doi.org/10.1016/j.phrs.2017.10.011

Alqahtani F, Aleanizy F, El Tahir E, Alhabib H, Alsaif R, Shazly G et al (2020) Antibacterial activity of chitosan nanoparticles against pathogenic N. gonorrhoea. Int J Nanomed 15:7877–7887. https://doi.org/10.2147/IJN.S272736

Ananth A, Dharaneedharan S, Heo M-S, Mok YS (2015) Copper oxide nanomaterials: synthesis, characterization and structure-specific antibacterial performance. Chem Eng J 262:179–188. https://doi.org/10.1016/j.cej.2014.09.083

Arciola CR, Campoccia D, Montanaro L (2018) Implant infections: adhesion, biofilm formation and immune evasion. Nat Rev Microbiol 16(7):397–409. https://doi.org/10.1038/s41579-018-0019-y

Arias LS, Pessan JP, Vieira APM, Lima TMT, Delbem ACB, Monteiro DR (2018) Iron oxide nanoparticles for biomedical applications: a perspective on synthesis, drugs, antimicrobial activity, and toxicity. Antibiotics 7(2). https://doi.org/10.3390/antibiotics7020046

Arora B, Murar M, Dhumale V (2015) Antimicrobial potential of TiO2 nanoparticles against MDR Pseudomonas aeruginosa. J Exp Nanosci 10(11):819–827. https://doi.org/10.1080/17458080.2014.902544

Arzanlou M, Chai WC, Venter H (2017a) Intrinsic, adaptive and acquired antimicrobial resistance in Gram-negative bacteria. Essays Biochem 61(1):49–59. https://doi.org/10.1042/EBC20160063

Arzanlou M, Chai Wern C, Venter H (2017b) Intrinsic, adaptive and acquired antimicrobial resistance in Gram-negative bacteria. Essays Biochem 61(1):49–59. https://doi.org/10.1042/ebc20160063

Aunkor MTH, Raihan T, Prodhan SH, Metselaar HSC, Malik SUF, Azad AK (2020) Antibacterial activity of graphene oxide nanosheet against multidrug resistant superbugs isolated from infected patients. R Soc Open Sci 7(7):200640. https://doi.org/10.1098/rsos.200640

Awaad MHH, Moustafa KME, Zoulfakar SA, Elhalawany MS, Mohammed FF, El-Refay RM et al (2021) The role of silver nanoparticles in the reluctance of colisepticemia in broiler chickens. J Appl Poult Res 30(2):100155. https://doi.org/10.1016/j.japr.2021.100155

Azeredo da Silveira S, Perez A (2017) Improving the fate of severely infected patients: the promise of anti-toxin treatments and superiority trials. Expert Rev Anti-Infect Ther 15(11):973–975. https://doi.org/10.1080/14787210.2017.1400908

Baker S, Pasha A, Satish S (2017) Biogenic nanoparticles bearing antibacterial activity and their synergistic effect with broad spectrum antibiotics: emerging strategy to combat drug resistant pathogens. Saudi Pharm J 25(1):44–51. https://doi.org/10.1016/j.jsps.2015.06.011

Baptista PV, McCusker MP, Carvalho A, Ferreira DA, Mohan NM, Martins M et al (2018) Nano-strategies to fight multidrug resistant bacteria—"a battle of the titans". Front Microbiol 9(1441). https://doi.org/10.3389/fmicb.2018.01441

Berger-Bachi B (1994) Expression of resistance to methicillin. Trends Microbiol 2(10):389–393. https://doi.org/10.1016/0966-842x(94)90617-3

Berlanga M, Guerrero R (2016) Living together in biofilms: the microbial cell factory and its biotechnological implications. Microb Cell Factories 15(1):165. https://doi.org/10.1186/s12934-016-0569-5

Blair KM, Turner L, Winkelman JT, Berg HC, Kearns DB (2008) A molecular clutch disables flagella in the Bacillus subtilis biofilm. Science 320(5883):1636–1638. https://doi.org/10.1126/science.1157877

Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ (2015) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13(1):42–51. https://doi.org/10.1038/nrmicro3380

Borkow G, Gabbay J (2009) Copper, an ancient remedy returning to fight microbial, fungal and viral infections. Curr Chem Biol 3(3):272–278

Brown AN, Smith K, Samuels TA, Lu J, Obare SO, Scott ME (2012) Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of Pseudomonas aeruginosa and Enterobacter aerogenes and methicillin-resistant Staphylococcus aureus. Appl Environ Microbiol 78(8):2768–2774. https://doi.org/10.1128/AEM.06513-11

Bruinenberg P, Blanchard JD, Cipolla DC, Dayton F, Mudumba S, Gonda I (eds) (2010) Inhaled liposomal ciprofloxacin: once a day management of respiratory infections. Respiratory drug delivery. Davis Healthcare International Publishing, River Grove, Orlando

Butler MT, Wang Q, Harshey RM (2010) Cell density and mobility protect swarming bacteria against antibiotics. Proc Natl Acad Sci U S A 107(8):3776–3781. https://doi.org/10.1073/pnas.0910934107

Cardozo VF, Lancheros CA, Narciso AM, Valereto EC, Kobayashi RK, Seabra AB et al (2014) Evaluation of antibacterial activity of nitric oxide-releasing polymeric particles against Staphylococcus aureus and Escherichia coli from bovine mastitis. Int J Pharm 473(1–2):20–29. https://doi.org/10.1016/j.ijpharm.2014.06.051

Chaudhari SP, Kalorey DR, Awandkar SP, Kurkure NV, Narang R, Kashyap RS et al (2021) Journey towards National Institute of One Health in India. Indian J Med Res 153(3):320–326. https://doi.org/10.4103/ijmr.IJMR_636_21

Chen G, Deng H, Song X, Lu M, Zhao L, Xia S et al (2017) Reactive oxygen species-responsive polymeric nanoparticles for alleviating sepsis-induced acute liver injury in mice. Biomaterials 144:30–41. https://doi.org/10.1016/j.biomaterials.2017.08.008

Chen X, Liu Y, Lin A, Huang N, Long L, Gang Y et al (2018) Folic acid-modified mesoporous silica nanoparticles with pH-responsiveness loaded with Amp for an enhanced effect against antidrug-resistant bacteria by overcoming efflux pump systems. Biomater Sci 6(7):1923–1935. https://doi.org/10.1039/C8BM00262B

Chen Q, Shah KN, Zhang F, Salazar AJ, Shah PN, Li R et al (2019a) Minocycline and silver dual-loaded polyphosphoester-based nanoparticles for treatment of resistant Pseudomonas aeruginosa. Mol Pharm 16(4):1606–1619. https://doi.org/10.1021/acs.molpharmaceut.8b01288

Chen W, Cheng F, Swing CJ, Xia S, Zhang X (2019b) Modulation effect of core-wall ratio on the stability and antibacterial activity of cinnamaldehyde liposomes. Chem Phys Lipids 223:104790. https://doi.org/10.1016/j.chemphyslip.2019.104790

Dakal TC, Kumar A, Majumdar RS, Yadav V (2016) Mechanistic basis of antimicrobial actions of silver nanoparticles. Front Microbiol 7:1831. https://doi.org/10.3389/fmicb.2016.01831

Deka RP, Magnusson U, Grace D, Lindahl J (2018) Bovine brucellosis: prevalence, risk factors, economic cost and control options with particular reference to India- a review. Infect Ecol Epidemiol 8(1):1556548. https://doi.org/10.1080/20008686.2018.1556548

Dhama K, Karthik K, Tiwari R, Shabbir MZ, Barbuddhe S, Malik SV et al (2015) Listeriosis in animals, its public health significance (food-borne zoonosis) and advances in diagnosis and control: a comprehensive review. Vet Q 35(4):211–235. https://doi.org/10.1080/01652176.2015.1063023

Dolansky J, Henke P, Mala Z, Zarska L, Kubat P, Mosinger J (2018) Antibacterial nitric oxide- and singlet oxygen-releasing polystyrene nanoparticles responsive to light and temperature triggers. Nanoscale 10(5):2639–2648. https://doi.org/10.1039/c7nr08822a

dos Santos MM, Queiroz MJ, Baptista PV (2012) Enhancement of antibiotic effect via gold:silver-alloy nanoparticles. J Nanopart Res 14(5):859. https://doi.org/10.1007/s11051-012-0859-8

Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV et al (2018) Multidrug efflux pumps: structure, function and regulation. Nat Rev Microbiol 16(9):523–539. https://doi.org/10.1038/s41579-018-0048-6

Duraisamy Y (2016) Strength and stiffness improvement of bio-cemented Sydney sand

Ejaz S, Ihsan A, Noor T, Shabbir S, Imran M (2020) Mannose functionalized chitosan nanosystems for enhanced antimicrobial activity against multidrug resistant pathogens. Polym Test 91:106814. https://doi.org/10.1016/j.polymertesting.2020.106814

El Zowalaty ME, Hussein Al Ali SH, Husseiny MI, Geilich BM, Webster TJ, Hussein MZ (2015) The ability of streptomycin-loaded chitosan-coated magnetic nanocomposites to possess antimicrobial and antituberculosis activities. Int J Nanomed 10:3269–3274. https://doi.org/10.2147/IJN.S74469

Eleraky NE, Allam A, Hassan SB, Omar MM (2020) Nanomedicine fight against antibacterial resistance: an overview of the recent pharmaceutical innovations. Pharmaceutics 12(2). https://doi.org/10.3390/pharmaceutics12020142

El-Zamkan MA, Hendy BA, Diab HM, Marraiki N, Batiha GE, Saber H et al (2021) Control of virulent Listeria monocytogenes originating from dairy products and cattle environment using marine algal extracts, silver nanoparticles thereof, and quaternary disinfectants. Infect Drug Resist 14:2721–2739. https://doi.org/10.2147/IDR.S300593

Fakhri A, Tahami S, Naji M (2017) 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 169:21–26. https://doi.org/10.1016/j.jphotobiol.2017.02.014

Ferreira C, Pereira A, Melo L, Simões M (2010) Advances in industrial biofilm control with micro-nanotechnology. In: Current research, technology and education topics in applied microbiology and microbial biotechnology, vol 2. Formatex. Badajoz, Spain, pp 845–854

Friedman AD, Kim D, Liu R (2015) Highly stable aptamers selected from a 2′-fully modified fGmH RNA library for targeting biomaterials. Biomaterials 36:110–123. https://doi.org/10.1016/j.biomaterials.2014.08.046

Fux CA, Costerton JW, Stewart PS, Stoodley P (2005) Survival strategies of infectious biofilms. Trends Microbiol 13(1):34–40. https://doi.org/10.1016/j.tim.2004.11.010

Gabrielyan L, Hakobyan L, Hovhannisyan A, Trchounian A (2019) Effects of iron oxide (Fe3 O4) nanoparticles on Escherichia coli antibiotic-resistant strains. J Appl Microbiol 126(4):1108–1116. https://doi.org/10.1111/jam.14214

Gao W, Thamphiwatana S, Angsantikul P, Zhang L (2014) Nanoparticle approaches against bacterial infections. Wiley Interdiscip Rev Nanomed Nanobiotechnol 6(6):532–547. https://doi.org/10.1002/wnan.1282

Gao W, Chen Y, Zhang Y, Zhang Q, Zhang L (2018) Nanoparticle-based local antimicrobial drug delivery. Adv Drug Deliv Rev 127:46–57. https://doi.org/10.1016/j.addr.2017.09.015

Gehring J, Trepka B, Klinkenberg N, Bronner H, Schleheck D, Polarz S (2016) Sunlight-triggered nanoparticle synergy: teamwork of reactive oxygen species and nitric oxide released from mesoporous organosilica with advanced antibacterial activity. J Am Chem Soc 138(9):3076–3084. https://doi.org/10.1021/jacs.5b12073

Geilich BM, Gelfat I, Sridhar S, van de Ven AL, Webster TJ (2017) Superparamagnetic iron oxide-encapsulating polymersome nanocarriers for biofilm eradication. Biomaterials 119:78–85. https://doi.org/10.1016/j.biomaterials.2016.12.011

Ghasemi F, Jalal R (2016) Antimicrobial action of zinc oxide nanoparticles in combination with ciprofloxacin and ceftazidime against multidrug-resistant Acinetobacter baumannii. J Glob Antimicrob Resist 6:118–122. https://doi.org/10.1016/j.jgar.2016.04.007

Gholipourmalekabadi M, Mobaraki M, Ghaffari M, Zarebkohan A, Omrani VF, Urbanska AM et al (2017) Targeted drug delivery based on gold nanoparticle derivatives. Curr Pharm Des 23(20):2918–2929. https://doi.org/10.2174/1381612823666170419105413

Grumezescu AM, Gestal MC, Holban AM, Grumezescu V, Vasile BS, Mogoanta L et al (2014) Biocompatible Fe3O4 increases the efficacy of amoxicillin delivery against Gram-positive and Gram-negative bacteria. Molecules 19(4):5013–5027. https://doi.org/10.3390/molecules19045013

Güncüm E, Işıklan N, Anlaş C, Ünal N, Bulut E, Bakırel T (2018) Development and characterization of polymeric-based nanoparticles for sustained release of amoxicillin – an antimicrobial drug. Artif Cells Nanomed Biotechnol. 46(sup2):964–973. https://doi.org/10.1080/21691401.2018.1476371

Gutiérrez-Santana JC, Toscano-Garibay JD, López-López M, Coria-Jiménez VR (2020) Aptamers coupled to nanoparticles in the diagnosis and treatment of microbial infections. Enfermedades Infecciosas y Microbiología Clínica (English Edition). https://doi.org/10.1016/j.eimce.2020.05.001

Hall JR, Rouillard KR, Suchyta DJ, Brown MD, Ahonen MJR, Schoenfisc MH (2020) Mode of nitric oxide delivery affects antibacterial action. ACS Biomater Sci Eng 6(1):433–441. https://doi.org/10.1021/acsbiomaterials.9b01384

Hasanzadeh Kafshgari M, Delalat B, Harding FJ, Cavallaro A, Makila E, Salonen J et al (2016) Antibacterial properties of nitric oxide-releasing porous silicon nanoparticles. J Mater Chem B 4(11):2051–2058. https://doi.org/10.1039/c5tb02551f

Haworth CS, Bilton D, Chalmers JD, Davis AM, Froehlich J, Gonda I et al (2019) Inhaled liposomal ciprofloxacin in patients with non-cystic fibrosis bronchiectasis and chronic lung infection with Pseudomonas aeruginosa (ORBIT-3 and ORBIT-4): two phase 3, randomised controlled trials. Lancet Respir Med 7(3):213–226. https://doi.org/10.1016/S2213-2600(18)30427-2

Hernandez-Delgadillo R, Velasco-Arias D, Diaz D, Arevalo-Nino K, Garza-Enriquez M, De la Garza-Ramos MA et al (2012) Zerovalent bismuth nanoparticles inhibit Streptococcus mutans growth and formation of biofilm. Int J Nanomedicine 7:2109–2113. https://doi.org/10.2147/IJN.S29854

Hetrick EM, Shin JH, Paul HS, Schoenfisch MH (2009) Anti-biofilm efficacy of nitric oxide-releasing silica nanoparticles. Biomaterials 30(14):2782–2789. https://doi.org/10.1016/j.biomaterials.2009.01.052

Holt HR, Bedi JS, Kaur P, Mangtani P, Sharma NS, Gill JPS et al (2021) Epidemiology of brucellosis in cattle and dairy farmers of rural Ludhiana, Punjab. PLoS Negl Trop Dis 15(3):e0009102

Hua S, de Matos MBC, Metselaar JM, Storm G (2018) Current trends and challenges in the clinical translation of nanoparticulate nanomedicines: pathways for translational development and commercialization. Front Pharmacol 9(790). https://doi.org/10.3389/fphar.2018.00790

Huang Y, Ren J, Qu X (2019) Nanozymes: classification, catalytic mechanisms, activity regulation, and applications. Chem Rev 119(6):4357–4412. https://doi.org/10.1021/acs.chemrev.8b00672

Ikeda Y, Shoji K, Feliciano CP, Saito S, Nagasaki Y (2018) Antioxidative nanoparticles significantly enhance therapeutic efficacy of an antibacterial therapy against Listeria monocytogenes infection. Mol Pharm 15(3):1126–1132. https://doi.org/10.1021/acs.molpharmaceut.7b00995

Imbuluzqueta E, Gamazo C, Lana H, Campanero MA, Salas D, Gil AG et al (2013) Hydrophobic gentamicin-loaded nanoparticles are effective against Brucella melitensis infection in mice. Antimicrob Agents Chemother 57(7):3326–3333. https://doi.org/10.1128/AAC.00378-13

Kalhapure RS, Jadhav M, Rambharose S, Mocktar C, Singh S, Renukuntla J et al (2017) pH-responsive chitosan nanoparticles from a novel twin-chain anionic amphiphile for controlled and targeted delivery of vancomycin. Colloids Surf B Biointerfaces 158:650–657. https://doi.org/10.1016/j.colsurfb.2017.07.049

Kankala RK, Lin WZ, Lee CH (2020) Combating antibiotic resistance through the synergistic effects of mesoporous silica-based hierarchical nanocomposites. Nanomaterials 10(3). https://doi.org/10.3390/nano10030597

Kanugala S, Jinka S, Puvvada N, Banerjee R, Kumar CG (2019) Phenazine-1-carboxamide functionalized mesoporous silica nanoparticles as antimicrobial coatings on silicone urethral catheters. Sci Rep 9(1):6198. https://doi.org/10.1038/s41598-019-42722-9

Kaur A, Preet S, Kumar V, Kumar R, Kumar R (2019) Synergetic effect of vancomycin loaded silver nanoparticles for enhanced antibacterial activity. Colloids Surf B Biointerfaces 176:62–69. https://doi.org/10.1016/j.colsurfb.2018.12.043

Khan MZ, Zahoor M (2018) An overview of brucellosis in cattle and humans, and its serological and molecular diagnosis in control strategies. Trop Med Infect Dis 3(2). https://doi.org/10.3390/tropicalmed3020065

Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R et al (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 10(9):597–602. https://doi.org/10.1016/S1473-3099(10)70143-2

Lai S, Tremblay J, Déziel E (2009) Swarming motility: a multicellular behaviour conferring antimicrobial resistance. Environ Microbiol 11(1):126–136. https://doi.org/10.1111/j.1462-2920.2008.01747.x

Lange A, Grzenia A, Wierzbicki M, Strojny-Cieslak B, Kalińska A, Gołębiewski M et al (2021) Silver and copper nanoparticles inhibit biofilm formation by mastitis pathogens. Animals 11(7):1884

Laterre PF, Colin G, Dequin PF, Dugernier T, Boulain T, Azeredo da Silveira S et al (2019) CAL02, a novel antitoxin liposomal agent, in severe pneumococcal pneumonia: a first-in-human, double-blind, placebo-controlled, randomised trial. Lancet Infect Dis 19(6):620–630. https://doi.org/10.1016/S1473-3099(18)30805-3

Latif-ur R, Shah A, Khan SB, Asiri AM, Hussain H, Han C et al (2015) Synthesis, characterization, and application of Au–Ag alloy nanoparticles for the sensing of an environmental toxin, pyrene. J Appl Electrochem 45(5):463–472. https://doi.org/10.1007/s10800-015-0807-2

Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N et al (2013) Antibiotic resistance-the need for global solutions. Lancet Infect Dis 13(12):1057–1098. https://doi.org/10.1016/S1473-3099(13)70318-9

Lee I, Han SW, Choi HJ, Kim K (2001) Nanoparticle-directed crystallization of calcium carbonate. Adv Mater 13(21):1617–1620. https://doi.org/10.1002/1521-4095(200111)13:21<1617::AID-ADMA1617>3.0.CO;2-W

Li H, Yao Q-Z, Wang F-P, Huang Y-R, Fu S-Q, Zhou G-T (2019a) Insights into the formation mechanism of vaterite mediated by a deep-sea bacterium Shewanella piezotolerans WP3. Geochim Cosmochim Acta 256:35–48. https://doi.org/10.1016/j.gca.2018.06.011

Li Y, Wu Y, Chen J, Wan J, Xiao C, Guan J et al (2019b) A simple glutathione-responsive turn-on theranostic nanoparticle for dual-modal imaging and chemo-photothermal combination therapy. Nano Lett 19(8):5806–5817. https://doi.org/10.1021/acs.nanolett.9b02769

Liang Z, Liu Y, Sun X, Lin J, Yao J, Song Y et al (2020) Immunoregulatory and antimicrobial activity of bovine neutrophil β-defensin-5-loaded PLGA nanoparticles against Mycobacterium bovis. Pharmaceutics 12(12):1172

Liu S, Hu M, Zeng TH, Wu R, Jiang R, Wei J et al (2012) Lateral dimension-dependent antibacterial activity of graphene oxide sheets. Langmuir 28(33):12364–12372. https://doi.org/10.1021/la3023908

Liu N, Chang Y, Feng Y, Cheng Y, Sun X, Jian H et al (2017) {101}-{001} Surface heterojunction-enhanced antibacterial activity of titanium dioxide nanocrystals under sunlight irradiation. ACS Appl Mater Interfaces 9(7):5907–5915. https://doi.org/10.1021/acsami.6b16373

Liu P, Wang Y, An L, Tian Q, Lin J, Yang S (2018) Ultrasmall WO3- x@gamma-poly-l-glutamic acid nanoparticles as a photoacoustic imaging and effective photothermal-enhanced chemodynamic therapy agent for cancer. ACS Appl Mater Interfaces 10(45):38833–38844. https://doi.org/10.1021/acsami.8b15678

Lueth P, Haughney SL, Binnebose AM, Mullis AS, Peroutka-Bigus N, Narasimhan B et al (2019) Nanotherapeutic provides dose sparing and improved antimicrobial activity against

Brucella melitensis infections. J Control Release 294:288–297. https://doi.org/10.1016/j.jconrel.2018.12.024

Luo Y, Hossain M, Wang C, Qiao Y, An J, Ma L et al (2013) Targeted nanoparticles for enhanced X-ray radiation killing of multidrug-resistant bacteria. Nanoscale 5(2):687–694. https://doi.org/10.1039/c2nr33154c

Ma Z, Garrido-Maestu A, Jeong KC (2017) Application, mode of action, and in vivo activity of chitosan and its micro- and nanoparticles as antimicrobial agents: a review. Carbohydr Polym 176:257–265. https://doi.org/10.1016/j.carbpol.2017.08.082

Ma W, Zhang T, Li R, Niu Y, Yang X, Liu J et al (2020) Bienzymatic synergism of vanadium oxide nanodots to efficiently eradicate drug-resistant bacteria during wound healing in vivo. J Colloid Interface Sci 559:313–323. https://doi.org/10.1016/j.jcis.2019.09.040

Mah TF (2012) Biofilm-specific antibiotic resistance. Future Microbiol 7(9):1061–1072. https://doi.org/10.2217/fmb.12.76

Majumdar S, Pal S (2017) Bacterial intelligence: imitation games, time-sharing, and long-range quantum coherence. J Cell Commun Signal 11(3):281–284. https://doi.org/10.1007/s12079-017-0394-6

Manayani DJ, Thomas D, Dryden KA, Reddy V, Siladi ME, Marlett JM et al (2007) A viral nanoparticle with dual function as an anthrax antitoxin and vaccine. PLoS Pathog 3(10):1422–1431. https://doi.org/10.1371/journal.ppat.0030142

Marangon CA, Martins VCA, Ling MH, Melo CC, Plepis AMG, Meyer RL et al (2020) Combination of rhamnolipid and chitosan in nanoparticles boosts their antimicrobial efficacy. ACS Appl Mater Interfaces 12(5):5488–5499. https://doi.org/10.1021/acsami.9b19253

Marianelli C, Armas F, Boniotti MB, Mazzone P, Pacciarini ML, Di Marco Lo Presti V (2015) Multiple drug-susceptibility screening in Mycobacterium bovis: new nucleotide polymorphisms in the embB gene among ethambutol susceptible strains. Int J Infect Dis 33:39–44. https://doi.org/10.1016/j.ijid.2014.12.043

Marsich E, Travan A, Donati I, Turco G, Bellomo F, Paoletti S (2012) Tissue-implant antimicrobial interfaces. Antimicrobial polymers. John Wiley & Sons, Inc, Hoboken, pp 379–428

Mihu MR, Cabral V, Pattabhi R, Tar MT, Davies KP, Friedman AJ et al (2017) Sustained nitric oxide-releasing nanoparticles interfere with methicillin-resistant Staphylococcus aureus adhesion and biofilm formation in a rat central venous catheter model. Antimicrob Agents Chemother 61(1). https://doi.org/10.1128/AAC.02020-16

Mohammed AN, Abdel Aziz SAA (2019) Novel approach for controlling resistant Listeria monocytogenes to antimicrobials using different disinfectants types loaded on silver nanoparticles (AgNPs). Environ Sci Pollut Res Int 26(2):1954–1961. https://doi.org/10.1007/s11356-018-3773-5

Molchanova N, Hansen PR, Franzyk H (2017) Advances in development of antimicrobial peptidomimetics as potential drugs. Molecules 22(9). https://doi.org/10.3390/molecules22091430

Mottais A, Berchel M, Le Gall T, Sibiril Y, d'Arbonneau F, Laurent V et al (2019) Antibacterial and transfection activities of nebulized formulations incorporating long n-alkyl chain silver N-heterocyclic carbene complexes. Int J Pharm 567:118500. https://doi.org/10.1016/j.ijpharm.2019.118500

Moyá B, Beceiro A, Cabot G, Juan C, Zamorano L, Alberti S et al (2012) Pan-β-lactam resistance development in pseudomonas aeruginosa clinical strains: molecular mechanisms, penicillin-binding protein profiles, and binding affinities. Antimicrob Agents Chemother 56(9):4771–4778. https://doi.org/10.1128/AAC.00680-12

Mullard A (2016) FDA approves antitoxin antibody. Nat Rev Drug Discov 15(12):811. https://doi.org/10.1038/nrd.2016.257

Munir MU, Ahmed A, Usman M, Salman S (2020) Recent advances in nanotechnology-aided materials in combating microbial resistance and functioning as antibiotics substitutes. Int J Nanomedicine 15:7329–7358. https://doi.org/10.2147/IJN.S265934

Munoz-Egea MC, Garcia-Pedrazuela M, Mahillo-Fernandez I, Esteban J (2016) Effect of antibiotics and antibiofilm agents in the ultrastructure and development of biofilms developed

by nonpigmented rapidly growing mycobacteria. Microb Drug Resist 22(1):1–6. https://doi.org/10.1089/mdr.2015.0124

Muthuchamy M, Govindan R, Shine K, Thangasamy V, Alharbi NS, Thillaichidambaram M et al (2020) Anti-biofilm investigation of graphene/chitosan nanocomposites against biofilm producing P. aeruginosa and K. pneumoniae. Carbohydr Polym 230:115646. https://doi.org/10.1016/j.carbpol.2019.115646

Mydin RBSMN, Nadhirah I, Ishak NN, Shaida N, Moshawih S, Siddiquee S (2018) Potential of calcium carbonate nanoparticles for therapeutic applications. Malaysian J Med Health Sci 14:2636–9346

Narang AS, Chang RK, Hussain MA (2013) Pharmaceutical development and regulatory considerations for nanoparticles and nanoparticulate drug delivery systems. J Pharm Sci 102(11):3867–3882. https://doi.org/10.1002/jps.23691

Nguyen TK, Selvanayagam R, Ho KKK, Chen R, Kutty SK, Rice SA et al (2016) Co-delivery of nitric oxide and antibiotic using polymeric nanoparticles. Chem Sci 7(2):1016–1027. https://doi.org/10.1039/c5sc02769a

Ni J, Liu Y, Hussain T, Li M, Liang Z, Liu T et al (2021) Recombinant ArgF PLGA nanoparticles enhances BCG induced immune responses against Mycobacterium bovis infection. Biomed Pharmacother 137:111341. https://doi.org/10.1016/j.biopha.2021.111341

Nikaido H (2009) Multidrug resistance in bacteria. Annu Rev Biochem 78(1):119–146. https://doi.org/10.1146/annurev.biochem.78.082907.145923

Omolo CA, Kalhapure RS, Jadhav M, Rambharose S, Mocktar C, Ndesendo VM et al (2017) Pegylated oleic acid: a promising amphiphilic polymer for nano-antibiotic delivery. Eur J Pharm Biopharm 112:96–108. https://doi.org/10.1016/j.ejpb.2016.11.022

Osman N, Omolo CA, Gannimani R, Waddad AY, Rambharose S, Mocktar C et al (2019) Novel fatty acid-based pH-responsive nanostructured lipid carriers for enhancing antibacterial delivery. J Drug Deliv Sci Technol 53:101125. https://doi.org/10.1016/j.jddst.2019.101125

Pan W-Y, Huang C-C, Lin T-T, Hu H-Y, Lin W-C, Li M-J et al (2016) Synergistic antibacterial effects of localized heat and oxidative stress caused by hydroxyl radicals mediated by graphene/iron oxide-based nanocomposites. Nanomedicine 12(2):431–438. https://doi.org/10.1016/j.nano.2015.11.014

Patra P, Mitra S, Debnath N, Pramanik P, Goswami A (2014) Ciprofloxacin conjugated zinc oxide nanoparticle: a camouflage towards multidrug resistant bacteria. Bull Mater Sci 37(2):199–206. https://doi.org/10.1007/s12034-014-0637-6

Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS et al (2018) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 16(1):71. https://doi.org/10.1186/s12951-018-0392-8

Pauter K, Szultka-Młyńska M, Buszewski B (2020) Determination and identification of antibiotic drugs and bacterial strains in biological samples. Molecules 25(11):2556

Pelgrift RY, Friedman AJ (2013) Nanotechnology as a therapeutic tool to combat microbial resistance. Adv Drug Deliv Rev 65(13–14):1803–1815. https://doi.org/10.1016/j.addr.2013.07.011

Ponce CA, Chabe M, George C, Cardenas A, Duran L, Guerrero J et al (2017) High prevalence of Pneumocystis jirovecii dihydropteroate synthase gene mutations in patients with a first episode of Pneumocystis pneumonia in Santiago, Chile, and clinical response to trimethoprim-sulfamethoxazole therapy. Antimicrob Agents Chemother 61(2). https://doi.org/10.1128/AAC.01290-16

Privett BJ, Broadnax AD, Bauman SJ, Riccio DA, Schoenfisch MH (2012) Examination of bacterial resistance to exogenous nitric oxide. Nitric Oxide 26(3):169–173. https://doi.org/10.1016/j.niox.2012.02.002

Ramalingam B, Parandhaman T, Das SK (2016) Antibacterial effects of biosynthesized silver nanoparticles on surface ultrastructure and nanomechanical properties of Gram-negative bacteria viz. Escherichia coli and Pseudomonas aeruginosa. ACS Appl Mater Interfaces 8(7):4963–4976. https://doi.org/10.1021/acsami.6b00161

Ramalingam V, Sundaramahalingam S, Rajaram R (2019) Size-dependent antimycobacterial activity of titanium oxide nanoparticles against Mycobacterium tuberculosis. J Mater Chem B 7(27):4338–4346. https://doi.org/10.1039/C9TB00784A

Ranjan S, Ramalingam C (2016) Titanium dioxide nanoparticles induce bacterial membrane rupture by reactive oxygen species generation. Environ Chem Lett 14(4):487–494. https://doi.org/10.1007/s10311-016-0586-y

Renu S, Han Y, Dhakal S, Lakshmanappa YS, Ghimire S, Feliciano-Ruiz N et al (2020) Chitosan-adjuvanted Salmonella subunit nanoparticle vaccine for poultry delivered through drinking water and feed. Carbohydr Polym 243:116434. https://doi.org/10.1016/j.carbpol.2020.116434

Rinaldi F, Hanieh PN, Sennato S, De Santis F, Forte J, Fraziano M et al (2021) Rifampicin-liposomes for Mycobacterium abscessus infection treatment: intracellular uptake and antibacterial activity evaluation. Pharmaceutics 13(7). https://doi.org/10.3390/pharmaceutics13071070

Rinu S, Queen MAJ, Udhaya PA (2020) Synthesis, structural characterization and antibacterial applications of calcium nanoparticles. J Adv Sci Res 11(1):83–87

Rizzato C, Torres J, Kasamatsu E, Camorlinga-Ponce M, Bravo MM, Canzian F et al (2019) Potential role of biofilm formation in the development of digestive tract cancer with special reference to Helicobacter pylori infection. Front Microbiol 10:846. https://doi.org/10.3389/fmicb.2019.00846

Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D, Hye Park C et al (2006) Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med 12(1):83–88. https://doi.org/10.1038/nm1347

Rolain JM, Parola P, Cornaglia G (2010) New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia? Clin Microbiol Infect 16(12):1699–1701. https://doi.org/10.1111/j.1469-0691.2010.03385.x

Rong F, Tang Y, Wang T, Feng T, Song J, Li P et al (2019) Nitric oxide-releasing polymeric materials for antimicrobial applications: a review. Antioxidants 8(11). https://doi.org/10.3390/antiox8110556

Rosen J, Landriscina A, Nosanchuk JD (2016) Nitric oxide–releasing nanoparticles as an antimicrobial therapeutic. In: Nanoscience in dermatology. Elsevier, Amsterdam, pp 127–134

Rossi-Fedele G, Roberts AP (2007) A preliminary study investigating the survival of tetracycline resistant Enterococcus faecalis after root canal irrigation with high concentrations of tetracycline. Int Endod J 40(10):772–777. https://doi.org/10.1111/j.1365-2591.2007.01285.x

Ruddaraju LK, Pammi SVN, Guntuku GS, Padavala VS, Kolapalli VRM (2020) A review on antibacterials to combat resistance: from ancient era of plants and metals to present and future perspectives of green nano technological combinations. Asian J Pharm Sci 15(1):42–59. https://doi.org/10.1016/j.ajps.2019.03.002

Sanyasi S, Majhi RK, Kumar S, Mishra M, Ghosh A, Suar M et al (2016) Polysaccharide-capped silver nanoparticles inhibit biofilm formation and eliminate multi-drug-resistant bacteria by disrupting bacterial cytoskeleton with reduced cytotoxicity towards mammalian cells. Sci Rep 6:24929. https://doi.org/10.1038/srep24929

Schairer DO, Chouake JS, Nosanchuk JD, Friedman AJ (2012) The potential of nitric oxide releasing therapies as antimicrobial agents. Virulence 3(3):271–279. https://doi.org/10.4161/viru.20328

Seabra AB, Manosalva N, de Araujo LB, Pelegrino MT, Brocchi M, Rubilar O et al (2017) Antibacterial activity of nitric oxide releasing silver nanoparticles. J Phys Conf Ser 838:012031. https://doi.org/10.1088/1742-6596/838/1/012031

Selvaraj V, Manne ND, Arvapalli R, Rice KM, Nandyala G, Fankenhanel E et al (2015) Effect of cerium oxide nanoparticles on sepsis induced mortality and NF-kappaB signaling in cultured macrophages. Nanomedicine 10(8):1275–1288. https://doi.org/10.2217/nnm.14.205

Selvarajan V, Obuobi S, Ee PLR (2020) Silica nanoparticles—a versatile tool for the treatment of bacterial infections. Front Chem 8(602). https://doi.org/10.3389/fchem.2020.00602

Sharma D, Misba L, Khan AU (2019) Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control 8(1):76. https://doi.org/10.1186/s13756-019-0533-3

Sharun K, Dhama K, Tiwari R, Gugjoo MB, Iqbal Yatoo M, Patel SK et al (2021) Advances in therapeutic and managemental approaches of bovine mastitis: a comprehensive review. Vet Q 41(1):107–136. https://doi.org/10.1080/01652176.2021.1882713

Shi B, Yan Q, Tang J, Xin K, Zhang J, Zhu Y et al (2018) Hydrogen sulfide-activatable second near-infrared fluorescent nanoassemblies for targeted photothermal cancer therapy. Nano Lett 18(10):6411–6416. https://doi.org/10.1021/acs.nanolett.8b02767

Siddiqi KS, Ur Rahman A, Tajuddin, Husen A (2018) Properties of zinc oxide nanoparticles and their activity against microbes. Nanoscale Res Lett 13(1):141. https://doi.org/10.1186/s11671-018-2532-3

Singh B, Prasad S, Verma MR, Sinha D (2014) Estimation of economic losses due to haemorrhagic septicaemia in cattle and buffaloes in India. Agric Econ Res Rev 27(2):271–279

Singh R, Nawale L, Arkile M, Wadhwani S, Shedbalkar U, Chopade S et al (2016) Phytogenic silver, gold, and bimetallic nanoparticles as novel antitubercular agents. Int J Nanomedicine 11:1889–1897. https://doi.org/10.2147/IJN.S102488

Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM et al (2015) Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nanomicro Lett 7(3):219–242. https://doi.org/10.1007/s40820-015-0040-x

Song MY, Jurng J, Park Y-K, Kim BC (2016) An aptamer cocktail-functionalized photocatalyst with enhanced antibacterial efficiency towards target bacteria. J Hazard Mater 318:247–254. https://doi.org/10.1016/j.jhazmat.2016.07.016

Sreedharan SM, Singh R (2019) Ciprofloxacin functionalized biogenic gold nanoflowers as nano-antibiotics against pathogenic bacterial strains. Int J Nanomedicine 14:9905–9916. https://doi.org/10.2147/IJN.S224488

Ssekatawa K, Byarugaba DK, Kato CD, Ejobi F, Tweyongyere R, Lubwama M et al (2020) Nanotechnological solutions for controlling transmission and emergence of antimicrobial-resistant bacteria, future prospects, and challenges: a systematic review. J Nanopart Res 22(5):117. https://doi.org/10.1007/s11051-020-04817-7

Stewart PS (2002) Mechanisms of antibiotic resistance in bacterial biofilms. Int J Med Microbiol 292(2):107–113. https://doi.org/10.1078/1438-4221-00196

Sun D-S, Kau J-H, Huang H-H, Tseng Y-H, Wu W-S, Chang H-H (2016) Antibacterial properties of visible-light-responsive carbon-containing titanium dioxide photocatalytic nanoparticles against anthrax. Nano 6(12):237

Sushma B, Shedole S, Suresh KP, Leena G, Patil SS, Srikantha G (2021) An estimate of global anthrax prevalence in livestock: a meta-analysis. Vet World 14(5):1263–1271. https://doi.org/10.14202/vetworld.2021.1263-1271

Tang S, Zheng J (2018) Antibacterial activity of silver nanoparticles: structural effects. Adv Healthc Mater 7(13):e1701503. https://doi.org/10.1002/adhm.201701503

Teli MK, Mutalik S, Rajanikant GK (2010) Nanotechnology and nanomedicine: going small means aiming big. Curr Pharm Des 16(16):1882–1892. https://doi.org/10.2174/138161210791208992

Thirumalaivasan N, Venkatesan P, Lai P-S, Wu S-P (2019) In vitro and in vivo approach of hydrogen-sulfide-responsive drug release driven by azide-functionalized mesoporous silica nanoparticles. ACS Appl Bio Mater 2(9):3886–3896. https://doi.org/10.1021/acsabm.9b00481

Tinkle S, McNeil SE, Muhlebach S, Bawa R, Borchard G, Barenholz YC et al (2014) Nanomedicines: addressing the scientific and regulatory gap. Ann N Y Acad Sci 1313:35–56. https://doi.org/10.1111/nyas.12403

Tonoyan L, Fleming GTA, Mc Cay PH, Friel R, O'Flaherty V (2017) Antibacterial potential of an antimicrobial agent inspired by peroxidase-catalyzed systems. Front Microbiol 8:680. https://doi.org/10.3389/fmicb.2017.00680

Ucak S, Sudagidan M, Borsa BA, Mansuroglu B, Ozalp VC (2020) Inhibitory effects of aptamer targeted teicoplanin encapsulated PLGA nanoparticles for Staphylococcus aureus strains. World J Microbiol Biotechnol 36(5):69. https://doi.org/10.1007/s11274-020-02845-y

Valappil SP (2018) Nanosystems and antibacterial applications. In: Drug delivery nanosystems for biomedical applications. Elsevier, Amsterdam, pp 75–90

Vallabani NVS, Vinu A, Singh S, Karakoti A (2020) Tuning the ATP-triggered pro-oxidant activity of iron oxide-based nanozyme towards an efficient antibacterial strategy. J Colloid Interface Sci 567:154–164. https://doi.org/10.1016/j.jcis.2020.01.099

Van Meervenne E, De Weirdt R, Van Coillie E, Devlieghere F, Herman L, Boon N (2014) Biofilm models for the food industry: hot spots for plasmid transfer? Pathog Dis 70(3):332–338. https://doi.org/10.1111/2049-632X.12134

Venkatasubbu GD, Baskar R, Anusuya T, Seshan CA, Chelliah R (2016) Toxicity mechanism of titanium dioxide and zinc oxide nanoparticles against food pathogens. Colloids Surf B Biointerfaces 148:600–606. https://doi.org/10.1016/j.colsurfb.2016.09.042

Wang F, Tang R, Yu H, Gibbons PC, Buhro WE (2008) Size- and shape-controlled synthesis of bismuth nanoparticles. Chem Mater 20(11):3656–3662. https://doi.org/10.1021/cm8004425

Wang Y, Ding X, Chen Y, Guo M, Zhang Y, Guo X et al (2016) Antibiotic-loaded, silver core-embedded mesoporous silica nanovehicles as a synergistic antibacterial agent for the treatment of drug-resistant infections. Biomaterials 101:207–216. https://doi.org/10.1016/j.biomaterials.2016.06.004

Wang C, Zhang K, Zhou Z, Li Q, Shao L, Hao RZ et al (2017a) Vancomycin-modified Fe3O4@SiO2@Ag microflowers as effective antimicrobial agents. Int J Nanomedicine 12:3077–3094. https://doi.org/10.2147/IJN.S132570

Wang J, Wu G, Liu X, Sun G, Li D, Wei H (2017b) A decomposable silica-based antibacterial coating for percutaneous titanium implant. Int J Nanomedicine 12:371–379. https://doi.org/10.2147/IJN.S123622

Wang L, Hu C, Shao L (2017c) The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine 12:1227–1249. https://doi.org/10.2147/IJN.S121956

Wang S, Mao B, Wu M, Liang J, Deng L (2018) Influence of aptamer-targeted antibiofilm agents for treatment of Pseudomonas aeruginosa biofilms. Antonie Van Leeuwenhoek 111(2):199–208. https://doi.org/10.1007/s10482-017-0941-4

Wassel MO, Khattab MA (2017) Antibacterial activity against Streptococcus mutans and inhibition of bacterial induced enamel demineralization of propolis, miswak, and chitosan nanoparticles based dental varnishes. J Adv Res 8(4):387–392. https://doi.org/10.1016/j.jare.2017.05.006

Weiss S, Kobiler D, Levy H, Pass A, Ophir Y, Rothschild N et al (2011) Antibiotics cure anthrax in animal models. Antimicrob Agents Chemother 55(4):1533–1542. https://doi.org/10.1128/AAC.01689-10

Willyard C (2017) The drug-resistant bacteria that pose the greatest health threats. Nature 543(7643):15. https://doi.org/10.1038/nature.2017.21550

Wu X, Tan S, Xing Y, Pu Q, Wu M, Zhao JX (2017) Graphene oxide as an efficient antimicrobial nanomaterial for eradicating multi-drug resistant bacteria in vitro and in vivo. Colloids Surf B Biointerfaces 157:1–9. https://doi.org/10.1016/j.colsurfb.2017.05.024

Wyszogrodzka G, Marszałek B, Gil B, Dorożyński P (2016) Metal-organic frameworks: mechanisms of antibacterial action and potential applications. Drug Discov Today 21(6):1009–1018. https://doi.org/10.1016/j.drudis.2016.04.009

Xia MY, Xie Y, Yu CH, Chen GY, Li YH, Zhang T et al (2019) Graphene-based nanomaterials: the promising active agents for antibiotics-independent antibacterial applications. J Control Release 307:16–31. https://doi.org/10.1016/j.jconrel.2019.06.011

Xie S, Yang F, Tao Y, Chen D, Qu W, Huang L et al (2017) Enhanced intracellular delivery and antibacterial efficacy of enrofloxacin-loaded docosanoic acid solid lipid nanoparticles against intracellular Salmonella. Sci Rep 7(1):41104. https://doi.org/10.1038/srep41104

Yang W-T, Ke C-Y, Wu W-T, Lee R-P, Tseng Y-H (2019) Effective treatment of bovine mastitis with intramammary infusion of *Angelica dahurica* and *Rheum officinale* extracts. Evid Based Complement Alternat Med 2019:7242705. https://doi.org/10.1155/2019/7242705

Yashchenok AM, Borisova D, Parakhonskiy BV, Masic A, Pinchasik B, Möhwald H et al (2012) Nanoplasmonic smooth silica versus porous calcium carbonate bead biosensors for detection of biomarkers. Ann Phys 524(11):723–732. https://doi.org/10.1002/andp.201200158

Yeom J-H, Lee B, Kim D, Lee J-K, Kim S, Bae J et al (2016) Gold nanoparticle-DNA aptamer conjugate-assisted delivery of antimicrobial peptide effectively eliminates intracellular Salmonella enterica serovar Typhimurium. Biomaterials 104:43–51. https://doi.org/10.1016/j.biomaterials.2016.07.009

Yin W, Yu J, Lv F, Yan L, Zheng LR, Gu Z et al (2016) Functionalized nano-MoS2 with peroxidase catalytic and near-infrared photothermal activities for safe and synergetic wound antibacterial applications. ACS Nano 10(12):11000–11011. https://doi.org/10.1021/acsnano.6b05810

Yuan Y-G, Peng Q-L, Gurunathan S (2017) Effects of silver nanoparticles on multiple drug-resistant strains of Staphylococcus aureus and Pseudomonas aeruginosa from mastitis-infected goats: an alternative approach for antimicrobial therapy. Int J Mol Sci 18(3):569

Zhang L, Mah TF (2008) Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. J Bacteriol 190(13):4447–4452. https://doi.org/10.1128/JB.01655-07

Zhang K, Zhang J, Xi Z, Li LY, Gu X, Zhang QZ et al (2017) A new H2S-specific near-infrared fluorescence-enhanced probe that can visualize the H2S level in colorectal cancer cells in mice. Chem Sci 8(4):2776–2781. https://doi.org/10.1039/c6sc05646f

Zhao Y, Ye C, Liu W, Chen R, Jiang X (2014) Tuning the composition of AuPt bimetallic nanoparticles for antibacterial application. Angew Chem 53(31):8127–8131. https://doi.org/10.1002/anie.201401035

Zhao X, Jia Y, Dong R, Deng J, Tang H, Hu F et al (2020) Bimetallic nanoparticles against multi-drug resistant bacteria. Chem Commun 56(74):10918–10921. https://doi.org/10.1039/d0cc03481a