4 Antimicrobial Activity of Metallic Nanoparticles Using Prokaryotic Model Organisms

Preeti C. Sangave, Nivedita M. Matkar, and Vasanti Suvarna

Abstract

Infectious diseases has remained one of the leading causes of morbidity and mortality in the past decade. The problem has further exacerbated due to the lack of newer antibiotics and emergence of antimicrobial drug resistance among the pathogens. Bacteria have evolved diverse mechanisms which make them resistant to many antimicrobials simultaneously. These alarming situations have triggered worldwide initiatives in the direction of developing novel strategies, effective antimicrobial agents, and efficient targeting systems. One of the fields which holds promise to provide solutions as efficient antibacterial agents is nanotechnology. The field of nanotechnology is rapidly evolving with more applications being developed in the pharmaceutical and biomedical domains. Nanoparticles such as metallic and metal-oxide, have gained tremendous attention owing to intrinsic antibacterial properties. These properties have been further enhanced by their surface functionalization approaches. They are being explored as delivery agents to inhibit bacterial population and also to combat drug resistance mechanisms in pathogens. The present chapter summarizes recent scientific advances on metal, metal oxide nanoparticles, and nanocomposites-preparation methods along with their antibacterial potential evaluated in prokaryotic bacterial model systems.

P. C. Sangave (✉)
School of Pharmacy & Technology Management, SVKM's NMIMS, MPTP, Shirpur, India
e-mail: preeti.sangave@nmims.edu

N. M. Matkar
University of Mumbai, Mumbai, India

V. Suvarna
SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Mumbai, India
e-mail: vasanti.suvarna@bncp.ac.in

D. B. Siddhardha et al. (eds.), *Model Organisms to Study Biological Activities and Toxicity of Nanoparticles*, https://doi.org/10.1007/978-981-15-1702-0_4

Keywords
Metal nanoparticles · Metal oxide nanoparticles · Nanoparticle conjugates · Bacteria · MDR

4.1 Introduction

Metals whose dimensions fall in the range of 1–100 nm with respect to their length, width, or thickness are categorized as metal nanoparticles. Various kinds of nanomaterials are produced using physical, chemical, and biological (green synthesis) methods. With the unique properties acquired due to their dimensions, nanomaterials find varied applications in the fields of electronics, cosmetics and personal care, food & beverage industries, pharmaceutical & healthcare, and other industries such as textile, wastewater treatment, aerospace, glass, & paints (Willems 2005). Based on the metal, the global metal nanoparticles market has been segmented into silver, gold, iron, copper, platinum, graphite, titanium, and others (Willems 2005).

Owing to their antimicrobial properties, gold and silver nanoparticles are used in pharmaceutical & healthcare segment (Willems 2005). Silver nanoparticles have accounted for the largest share of the metal nanoparticles market. Recently they were valued over USD 1.3 billion in 2017 and would continue to lead the market in the next 5 years. Silver metal's exceptional therapeutic antimicrobial properties against pathogens and other microorganisms attribute to its growing popularity, which is being extended to food packing industry (Willems 2005). Moreover, the application of metal nanoparticles in electrical & electronics industry is projected to impact the market. This segment is projected to grow at the second-highest rate in the next 5 years (Willems 2005).

Other metal nanoparticles such copper, zinc, and graphite are being used as catalysts in different chemical and electrochemical reactions. They are also being used in multilayered ceramic capacitors, in conductive layers, and wire printing. Carbon, graphite, and copper nanoparticles find applications as lubricants in engine oils to impart wear resistance and reduce friction (Willems 2005). The ever growing diverse applications of metal nanoparticles along with their engineered and improvised versions are further going to boost the entire metal nanoparticle segment.

Nanoparticles (NPs) owing to their extremely large surface area in proportion to their size gather strategic advantages over their normal-size counterparts. Decreasing and bringing the size of the material in the nano-range may provide higher activity at smaller doses, thereby decreasing the effective dose to control pathogens (Hazan et al. 2015). The use of nanostructure-based materials as potent antibacterials to combat bacterial infections and moreover control drug resistances among microbes has gained tremendous importance in recent years (Baranwal et al. 2018).

Soon after the antibiotics were put to clinical use as drugs to treat and combat microbial infections, a remarkable feature was being evidenced among microorganisms i.e., to persist, survive, adapt, and to evolve in the presence of antibiotics, a phenomenon now well known as antimicrobial resistance. Lack of new antimicrobials

together with the emergence and spread of multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Seil and Webster 2012) or vancomycin-resistant *Enterococcus* (VRE) (Hamilton and Wenlock 2016), are currently a global threat to public health and livestock ((Rudramurthy et al. 2016); (Baptista et al. 2018). These pressing issues have prompted research worldwide to develop novel antimicrobials along with development of effective delivery or targeting strategies. Approaches such as use of nanomaterials and their engineered versions are being currently explored as a plausible solution to overcome the growing problem of antibiotic resistance in microbes.

In this chapter, we present an overview on various metal nanoparticles and their forms used as antibacterial agents. Recent advances in the form of nanoparticle conjugates have also been summarized in the subsequent section. Though it is difficult to give a comprehensive overview and discuss all forms of metal NPs, representative methods used of synthesis for NPs along with their characterisitcs have been briefly summarized. Main focus has been to summarize recent findings on the antibacterial effect as a function of size, morphology. Also focus on the mechanism of activity of different metal NPs forms against multi-drug resistant (MDR) pathogens along with challenges and future prospects.

4.2 Metal and Metal Oxide Nanoparticles

Metals and their oxides in the form of nanoparticles, renowned for their intrinsic and potent antibacterial properties, include silver (Ag), iron oxide (Fe_3O_4), copper oxide (CuO), magnesium oxide, titanium oxide (TiO_2), zinc oxide (ZnO), and others (Hazan et al. 2015).

Different synthetic strategies are employed to generate metal NPs. Conventionally, these techniques have been classified into physical (by use of vapour deposition, microwave, ultrasound, etc.), chemical (by sol-gel, precipitation, etc.), and biological, economical and eco-friendly (by use of microbes, plant extract) methods (Willems 2005; Baranwal et al. 2018; Prasad et al. 2016).

4.3 Metal Nanoparticles and Antibacterial Mechanisms

NPs act in the complex growth environment comprising high protein contents, salts, polysaccharides, and the media components. These factors in turn can affect the stability of NPs causing them to aggregate at the concentrations at which they are studied (Schwegmann and Frimmel 2010). It appears that zero-valent metals greatly affect microorganisms during their growth phase. Due to the ionic properties and surface charge of metallic NPs, the forces of attraction & repulsion dominate the interaction between the NPs and the microorganisms. Such electrostatic interactions are integral and they promote adhesion of NPs to the cell surface of the microorganism to exert its toxic effect (Schwegmann and Frimmel 2010). A study demonstrated this phenomenon by the use of AuNPs that were tagged with anionic and cationic side chains.

Phosphatidylcholine and phosphatidylserine vesicles representing a reference system for cell membranes that carried overall negative charge and another preparation containing only phosphatidylcholine that had no net charge were used. The positively charged AuNPs lysed the negatively charged vesicles more efficiently than the one with no charge (Schwegmann and Frimmel 2010). For different metal NPs or their engineered forms, three broad antibacterial mechanisms have been proposed (1) by liberation of ions; (2) by generation of reactive oxidative species and (3) interaction of NPs with the cell membrane (Schwegmann and Frimmel 2010).

Once the metal NPs are brought in contact with the microbial cell, the metallic nanoparticles attach to the cell via transmembrane protein. Attachment to the membrane proteins triggers conformational changes in the protein, thus blocking the transport channels. Smaller sized NPs are found to be more efficient in causing this than the larger sized NPs. Larger surface area of the smaller NPs permit superior binding forces and better adhesion followed by internalization. After internalization the NPs undergo ionization within the cell that further damage intracellular structures causing cell death (Schwegmann and Frimmel 2010).

The other mechanism observed with use of metal NPs is the generation of reactive oxidative species (ROS) to cause the antibacterial effect. ROS are group of oxidants that are highly reactive but short-lived and they include hydrogen peroxide (H_2O_2), superoxide radicals (O_2^-), singlet oxygen ($^1O_2$), and hydroxyl radicals (•OH). Due to their reactive nature, they cause damage to the cellular components like DNA, RNA, ribosomes, proteins and also to peptidoglycan present in cell membrane. ROS acts by altering and halting the processes of transcription, translation, enzyme activity, or the electron transport chain (Schwegmann and Frimmel 2010). Further some metal atoms can cause damage to the enzyme structure and deactivate them by attaching to the thiol group of the enzyme. It has been proposed that they are also capable of attaching to the purine and pyrimidine bases. They too disrupt the hydrogen bonding between to the helical strands of DNA causing its structural damage (Baptista et al. 2018).

Expression studies using reverse transcription-qPCR techniques are being performed for a better understanding of the impact of metal NPs at the cellular level. Recent findings of such molecular mechanisms strongly indicate upregulation of different stress response genes in the microorganism. When subjected to MgO treatment, the expression of oxidative stress response genes such as *katA* (expressing catalase), *ahpC* (expressing alkyl hydroperoxide reductase), and *dps* (expressing bacterioferritin) were upregulated 44-, 5-, and 4- fold, respectively along with a 22-fold higher expression of general stress response gene *spoT* in *C. jejuni* cells (He et al. 2016).

Table 4.1 summarizes representative examples of metal NPs, their methods of synthesis, morphological characteristics and antibacterial effects.

Table 4.1 Representative metal nanoparticles, their forms, syntheses and antibacterial mechanisms

Metal/ Metal oxide NPs	Method of synthesis	Size	Shape	Model organisms	Antibacterial mechanism	Reference
Silver nanoparticles (AgNPs)	Nitrate-reducing *Bacillus subtilis L1* (KT266579.1)	~28.30 nm	Spherical	Gram-positive and Gram-negative organisms	DNA fragmentation, cell shrinkage and increase in surface roughness	Muthukrishnan et al. (2019)
Silver nanoparticles (AgNPs)	Extracellular filtrate of the epiphytic fungus *B. ochroleuca*	8–21 nm	Coated on fabrics of polyester and Cotton	*S. aureus* and *E. coli*, *C. albicans, C. glabrata,* and *C. parapsilosis, P. aeruginosa*	Inhibition of biofilm by *P. aeruginosa* and damage to the intracellular contents of other bacteria tested	Rodrigues et al. (2019)
Ag–TiO_2 nanocomposites	Silver nanoparticles deposited on titanium dioxide nanoparticles by a chemical reduction approach	15–30 ± 5 nm diameter	Spherical	*B. subtilis* and *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* and *C. albicans*	Anti-biofilm activity, more intensive for Gram-positive bacteria	Lungu et al. (2014)
Zinc oxide (ZnO) and titanium dioxide (TiO2) NPs	Commercially available	ZnO NPs <100 nm and TiO_2 NPs <50 nm	Spherical	Biofilm and non-biofilm-forming MRSA	Electromagnetic attraction between the microbe and treated surface, microbe oxidization and death	Jesline et al. (2015)
Titanium dioxide (TiO_2) nanoparticles	Aqueous TiO_2	–	–	*E. coli*, *P. aeruginosa*, *S. aureus*, *Enterococcus hirae*, and *Bacteroides fragilis*	Photo-inactivation & cellular damage	Tsuang et al. (2008)

(continued)

Table 4.1 (continued)

Metal/ Metal oxide NPs	Method of synthesis	Size	Shape	Model organisms	Antibacterial mechanism	Reference
Nano-tubular Cu arrays	Template-based electrodeposition	Height 13±1 mm and diameter 300 nm	Tubular	*S. aureus, E.coli*, *Shigella sonnei, Salmonella enterica, and Candida albicans*	Over 4 log reduction for *S. aureus* via cellular damage	Razeeb et al. (2014)
Nickel nanoparticles	*Ocimum sanctum* leaf extract	12 and 36 nm	Spherical	*E. coli*, *K. pneumoniae*, and *S. typhi*), *B. subtilis*, *S. epidermidis*, and fungi (*C. albicans*, *C. tropicalis, A. fumigatus*, *A. clavatus*, and *A. niger)*	ROS generation, protein leakage & damage	Jeyaraj Pandian et al. (2016)
Zinc oxide nanostructures	Zinc acetate, dihydrate and NaOH	Length of petal ~800 nm	Hexagonal nanorods assembling into flower shape	*Staphylococcus aureus* and *Escherichia coli*	Overall antimicrobial effect	Mohan Kumar et al. (2013)
Magnetic binary nanocomposites: (1) Ag@Fe3O4 (magnetite) type and (2) g-Fe2O3@Ag (maghemite) type	In situ chemical reduction of silver ions of a magnetic phase by maltose, polyacrylate molecules as spacer among iron oxide and silver NPs.	(1) Ag@ Fe3O4: 5 nm (2) g-Fe2O3@ Ag: 20 and 40 nm	Spherical geometry	Ten tested bacterial strains and four *Candida* species	Overall strong antibacterial and antifungal activities	Prucek et al. (2011)
CuO nanoparticles	A gel combustion method	20 nm	Spherical geometry	Gram-positive and -negative bacteria	Overall strong antibacterial activities	Azam et al. (2012)

4.4 Metal Nanoparticles as Antimicrobials

4.4.1 Silver Nanoparticles (AgNPs)

Among different metal NPs, silver nanoparticles (AgNPs) have gained wider usage in varied fields. Silver has been known since ancient times to possess antibacterial action (Baptista et al. 2018). At present there are over 100 consumer products that use AgNPs for its superior antibacterial properties (Baranwal et al. 2018). Conventional chemical or green synthesis approaches have been used for preparing AgNPs. AgNPs can cause damage to the bacterial cell by inducing disruption of cell wall, oxidation mechanisms, or formation of ROS species (Baptista et al. 2018).

Synthesis of silver NPs (AgNPs) with an aqueous solution of $AgNO_3$ in the presence of culture supernatant of phenol-degraded broth was reported by Otari et al. (2014). The resultant AgNPs showed strong antimicrobial effects against Gram-positive, Gram-negative, and fungal microorganisms tested (Otari et al. 2014). In a similar study, cell-free extract of *Citrobacter* spp., *Escherichia* spp., and *Pseudomonas* spp. isolates were used to synthesize AgNPs which exhibited strong antibacterial effect (Bogdanović et al. 2014). In another study, synthesis of a silver–clay nanohybrid was performed by sodium borohydride reduction, followed by calcination and UV- irradiation method to uniformly precipitate silver on clay platelets. The resultant nanohybrid structure showed synergistic antimicrobial activity with a magnitude fourfold higher than the silver particles alone (Girase et al. 2011). The studies using AgNPs have been reviewed (Baptista et al. 2018; Khan et al. 2016).

4.4.2 Iron Oxide Nanoparticles (Fe_xO_yNPs)

Iron oxide nanoparticles have also been explored for their antibacterial effects. Iron oxide nanoparticle (IONPs) with magnetite (Fe_3O_4) like atomic arrangement and carrying negative surface potential (n-IONP) were prepared by co-precipitation method. These NPs were coated with positively charged chitosan molecule and caused reversal of the surface potential of n-IONP, i.e. positive surface potential IONP (p-IONP). The nanocrystals of spherical shape and with 10–20 nm diameter were obtained. At the concentrations <50 μM of n-IONP a 30% decrease in viability was observed. IOPN (p-IONP) after appropriate coating resulted in higher 70% reduction in cell viability of *B. subtilis* and *E. coli* strains. The antibacterial effects were attributed to increased ROS production in the presence of p-IONP (Arakha et al. 2015).

Another study proposed a different method to generate iron oxide NPs. The magnetic iron oxide nanoparticles (IONPs) were initially coated with polyvinyl pyrrolidone conjugated catechol (PVP-CCDP) followed by deposition of silver nanoparticles (Ag NPs) onto PVP-CCDP-coated IONPs in the presence of catechol. The resultant NPs were of 72 nm size and demonstrated a strong antibacterial effect against the two model microbes *E. coli* and a high *S. aureus* (Mosaiab et al. 2013).

4.4.3 Copper- and Copper Oxide Nanoparticles (Cu- & CuO-NPs)

The activity of copper as antibacterial has been known since ancient period (Cioffi and Rai 2012). Copper demonstrates antibacterial effect due to its ability to donate & accept electrons and thus generate hydroxyl radical causing damage to cellular components like proteins and lipids (Sánchez-Sanhueza et al. 2016). Small and bare CuNPs prepared by reduction method were active against test bacteria by interacting with cell membrane in a concentration-dependent manner (Bogdanović et al. 2014).

Duffya et al. (2018) reported the effectiveness of copper oxide (CuO) nanoparticles against *Salmonella*. Also, evaluated a combined effect of silver (Ag) and CuO nanoparticles against *Campylobacter* isolated from poultry. The MIC against *Campylobacter* was in the order of Ag ≥ CuO ≥ ZnO nanoparticles. AgNPs were the most active against *Salmonella* (Duffy et al. 2018). In another study, authors reported the use of copper NPs as antibacterial against *E. coli* strain. The NP caused formation of ROS, protein oxidation, lipid peroxidation along with DNA damage (Chatterjee et al. 2014). The bioactivity of copper-based nanomaterials have been reviewed in literature (Cioffi and Rai 2012; Baptista et al. 2018).

4.4.4 Magnesium Oxide Nanoparticles NPs (MgO-NPs)

Importance of another inorganic material, magnesium oxide (MgO) in nano-form, is not less. It has many diverse applications in the field of adsorbents, toxic waste remediation, as additives in heavy fuel oils, catalysis, as catalyst supports, superconducting & ferroelectric thin films as the substrate, reflecting & anti-reflecting coatings, as superconductors and in lithium ion batteries. Though there are fewer reports, nano-forms of magnesium oxide (MgO-NPs) alone and in combination are being explored as antibacterial agent (Jin and He 2011). In a study, the use of magnesium oxide nanoparticles (MgO-NPs) alone and paired with other antimicrobials (ZnO-NPs & nisin) against *Salmonella* Stanley and *Escherichia coli* O157:H7 were evaluated. The MgO-NPs showed over 7 log reductions in bacterial counts. Only when combined with nisin, MgO-NPs showed synergistic effect. However, combination with ZnO-NPs did not show any enhancement of antibacterial effect of MgO-NPs. Scanning electron microscopy (SEM) images depicted cell membrane damage and leakage of intracellular contents in MgO-NPs-treated cells that finally lead to death of pathogen (Jin and He 2011).

One of the study evaluated antibacterial efficacy of MgO-NPs against three important foodborne pathogens *E. coli* O157:H7, *C. jejuni,* and *Salmonella enteritidis*. Results indicated that MgO-NPs with 20 nm diameter, a MIC of 2 mg/mL MgO-NPs was required against *C. jejuni* with 2 h exposure, whereas complete inhibition of both *E. coli* O157:H7 and *S. enteritidis* required minimum 8 mg/mL nanoparticles in 4 h. Evaluation of oxidative stress genes, membrane permeability, and hydrogen peroxide levels indicated interaction of nanoparticles with pathogens triggering cell membrane damage, oxidative stress, and ultimately causing cell death (He et al. 2016). MgO nanowires were synthesized of 6 nm diameter by

reaction between magnesium acetate with urea by a microwave hydrothermal technique. The obtained nanowires showed concentration-dependent antibacterial activity, against both *Bacillus* sp. and *E. coli*. The production of superoxide anions (O_2^-) in large quantities on the surface of MgO nanowires interacted with peptide linkages in the bacterial cell walls to finally destroy them (Al-Hazmi et al. 2012).

4.4.5 Titanium Dioxide Nanoparticles (TiO_2-NPs)

Titanium dioxide (TiO_2) NPs are produced in large quantities worldwide for use in a wide range of applications such as pigment and cosmetic products for their ultraviolet blocking ability (Trouiller et al. 2009). They have also been used for their photocatalytic properties for applications such as the removal of contaminants, & recently for disinfection of surfaces such as clothes/ glass, air and water remediation (Foster et al. 2011). Few reports have published the effects of photoactivated TiO_2 on microorganisms as potential antibacterial agent. They have been tested against bacteria, fungi, algae, and viruses. The killing mechanism involving production of ROS, degradation of the cell wall and membrane have been proposed. Titanium dioxide NPs decorated with Ag and Cu pronounced their antibacterial effects. The findings from such reports are reviewed further in detail in literature (Foster et al. 2011).

In one study, the commercially available zinc- and titanium dioxide nanoparticles showed considerable activity against biofilm-producing methicillin-resistant *S. aureus* isolates (Jesline et al. 2015). The antimicrobial activity of silver doped with titanium nanoparticles was determined in one study. Silver-coated nanoparticles of 20–25 nm size were effective among different sizes of nanoparticles and did not cause significant cytotoxicity (Martinez-Gutierrez et al. 2010).

4.4.6 Zinc Oxide Nanoparticles (ZnO-NPs)

Another category of metal oxide NPs that has gained recent interest as an antibacterial agent due to its nontoxic and biocompatible nature to human cells is zinc oxide (Sirelkhatim et al. 2015). Zinc oxide quantum dots (ZnO-QDs) as nanoparticles were evaluated against *S. enteritidis*, *L. monocytogenes*, and *E. coli* O157:H7. The ZnO-QDs were used as a powder, bound in a polystyrene film (ZnO-PS), or suspended in a polyvinylprolidone gel (ZnO-PVP). The ZnO-PVP caused a 6.0 log reduction of *E. coli* O157:H7 and a 5.3 log reduction of *L. monocytogenes* at 3.2mgZnO/mL concentration after 48 h of incubation. ZnO powder and ZnO-PVP showed remarkable antimicrobial activities against all three pathogens (Jin et al. 2009). ZnO antibacterial effect was more evident with the Gram-positive than the Gram-negative bacteria (Premanathan et al. 2011). The ZnO-NPs were prepared by sol-gel method and the effect of particle size & surface modification on its antibacterial activity was studied. Different reaction times were used to control the NPs' size and this was followed by the addition of (3-glycidyloxypropyl) trimethoxysilane (GPTMS) as a surface modifier. The smaller ZnO-NPs of the dimension 5 nm had significant

bactericidal activity when tested against *Staphylococcus aureus.* The smaller NPs were more efficient in causing damage to the bacterial cell wall than the larger NPs as confirmed by SEM studies (Lallo da Silva et al. 2019).

In a study, authors studied the expression of few important superfamily class efflux pump genes found in MDR *S. aureus* in the presence of ZnO nanoparticle conjugates. The ZnO nanoparticles (NPs) conjugated to thiosemicarbazide (TSC) under amine functionalization by glutamic acid (ZnO-Glu–TSC) and ciprofloxacin (CIP) were used for the study. The results showed significant 2–8-fold decrease in MIC of ZnO-Glu–TSC NPs compared to CIP alone. Moreover, the combination of ZnO-Glu–TSC NPs and CIP (at their ½ MIC) significantly attenuated the expression of efflux genes- *norA*, *norB*, *norC*, and *tet38* compared to the CIP alone (Nejabatdoust et al. 2019). The authors screened thirty-six metal ions to inhibit pyocyanin production and biofilm formation of *P. aeruginosa* in LB medium at a concentration of 1 mM. Of the metal ions evaluated, 10 metal ions, namely Fe^{2+}, Ag^{+}, Ga^{3+}, In^{3+}, Pt^{4+}, Cd^{2+}, CrO_4^{2-}, Co^{2+}, Zn^{2+}, and TeO_3^{2-}, inhibited pyocyanin production and thus biofilm formation. Of these metal ions, three ions (Pt^{4+}, Ag + and TeO_3^{2-}) showed antibacterial activity by abolishing cell growth while, Zn^{2+} ($ZnCl_2$) was found to be the most active in hindering production of pyocyanin and formation of biofilm without displaying bactericidal effect (Lee et al. 2014).

Another study supported the above findings in which zinc and ZnO nanoparticles significantly inhibited the formation of biofilm and pyocyanin production, *Pseudomonas* quinolone signal (PQS), pyochelin, and hemolytic activity of *P. aeruginosa* without affecting the growth of planktonic cells. Further, the expression studies showed that ZnO-NPs induced the zinc cation efflux pump *czc* operon along with other transcriptional regulators (porin gene *opdT* and type III repressor *ptrA*), but repressed the pyocyanin-related *phz* operon (Lee et al. 2014).

4.4.7 Metal/Metal Oxide Nanocomposites

Instability and tendency of aggregation that often lead to loss of nano-size and special properties are considered as significant drawbacks for nanomaterials. Therefore, it is essential to prevent the tendency of aggregation and to stabilize nanomaterials (Zare and Shabani 2016). The coating of nanoparticles, fabrication of nanocomposites, or surface modification of NPs are some of the techniques adopted to engineer the nanoparticles to retain or enhance their properties such as antibacterial effects.

Metal and metal-based NPs are used as such or are coated with doping organic or inorganic substances or chemical and biological agents such as surfactants, peptides and proteins, polymers, antibiotics, or other metallic counterparts. All which in turn enhance their properties and impart better stability to the suspension. The overall surface properties and their resultant activities are thus greatly dependent on the composition and nature of these coatings (Mukherjee et al. 2011). Figure 4.1 indicates different functionalization strategies adapted to surface modify the metal- and metal oxide nanoparticles for their further antimicrobial effect.

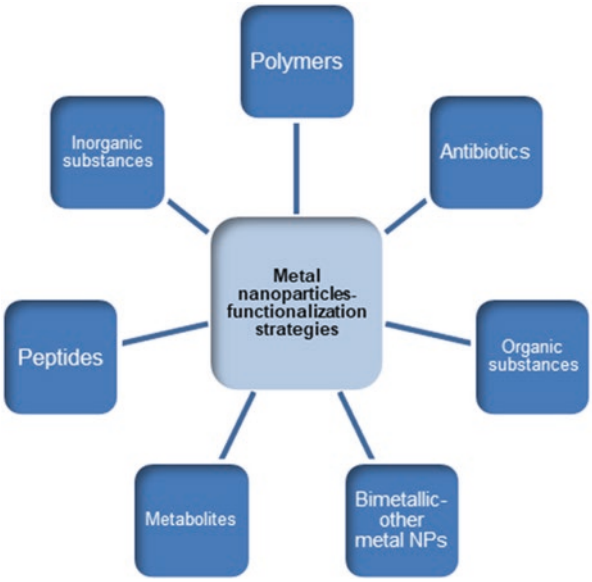

Fig. 4.1 Metal−/Metal oxide nanoparticle functionalization strategies

The authors demonstrated the photodynamic effect of Zn phthalocyanine-e-polylysine conjugates along with silver and gold nanoparticles (NPs) against *S. aureus*. The photoinactivation was observed to increase with concentration for conjugates. Phthalocyanines exhibited the singlet oxygen pathway causing photooxidative destruction of cellular components like sterols, peptides, and phospholipids (Nombona et al. 2012).

Zinc-based nano-metal organic frameworks (nMOFs) were fabricated and further explored for their antibacterial activities—both as mixture in the presence of antibiotics ampicillin & kanamycin and antibiotics alone. The nMOF/drug mixtures exhibited synergistic and additive effects compared to nMOFs or antibiotics alone, when tested against *E. coli*, *S. aureus*, *Staphylococcus lentus*, and *L. monocytogenes* (Bhardwaj et al. 2018).

Rhamnolipid (RL)-coated silver (Ag) and iron oxide (Fe_3O_4) NPs were prepared and evaluated against biofilm-forming *P. aeruginosa* and *S. aureus* strains. Silver of the dimension 35 nm and Fe_3O_4 NPs of the dimension 48 nm were used for this process. The presence of RLs on the NPs had significant impact in reducing the cell adhesion and biofilm forming properties of the pathogens by modifying the surface hydrophobicity of the bacterial strains. Moreover, the metal NPs generated ROS which potentiated antimicrobial effects along with RLs (Khalid et al. 2019).

Silver nanoparticles (Ag-NPs) anchored to graphene oxide (GO) were fabricated with different ratios and evaluated for their antibacterial potential. The activities of Ag-GO nanocomposites against *E. coli* and *S. aureus* model microorganisms were studied. Ag-GO nanocomposite was very effective at very low dosages of 14 μg/mL against *S. aureus* and 4 μg/mL against *E. coli*. The results also revealed Ag-GO

nanocomposite to be bactericidal causing cell wall disruption for *E. coli* while bacteriostatic for *S. aureus* by inhibiting cell division (Tang et al. 2013).

Authors reported the synthesis of a composite nanomaterial made of silver nanoparticle (AgNPs) implanted in nanofibers of poly-epsilon-caprolactone that were tested against different drug-resistant Gram-positive and Gram-negative microorganisms. Polycaprolactone-silver composites (PCL-AgNPs) showed dose-dependent increase in the antibacterial activity against *K. pneumoniae*, *E. coli*, *P. aeruginosa,* and *S. aureus* but not for *B. subtilis* and *S. mutans* (Pazos-Ortiz et al. 2017).

A study demonstrated the use of biocompatible halloysite nanoclay (HNTs) to intercalate silver nanoparticles (AgNPs) in the presence of curcumin as reducing agent. The resultant AgNPs showed good antimicrobial activity against *Bacillus cereus* and *Escherichia coli* cells (Sudhakar et al. 2017).

Polyvinyl alcohol (PVA) nanofibers containing Ag-NPs were synthesized and investigated for antibacterial activity in wound healing applications. The antibacterial activities of PVA/AgNO3 nanofibers against *S. aureus* and *K. pneumoniae* showed significant reduction of >99.9% after 18 h of incubation (Marega et al. 2015).

Dendritic Fe_3O_4 magnetic nanoadsorbents (DMNA) as spherical particles with 10 nm diameter were synthesized. At low concentration they showed around 97% inhibitory effect against *S. aureus* and good inhibitory activity against *B. subtilis*, *E. coli,* and *P. aeruginosa* strains (Singh and Bahadur 2019).

Sol-gel-based method was used to generate well-distributed hybrid copper nanostructures supported directly on the surface of the silica nanoparticles. These nanostructured particles formulated into a film that eliminated 99% of bio-burden at 0.01% in 6 h. These hybrid Cu-based nanoparticles liberated copper ions giving resultant effect against model organisms *S. aureus* and *E. coli* (Palza et al. 2015).

Graphene oxide was used as a carrier for metal- and metalloid-based nanoparticles (Zn, Cu, Mg, Ag, Se, AgP) to generate nanocomposites. These materials were evaluated for their antibacterial activity against *S. aureus*, MRSA, and *E. coli* strains as model organisms. A highest inhibition of 87.4% was observed with graphene oxide composite made with selenium nanoparticles versus control followed by silver and silver phosphate NPs against *S. aureus*. A dose-dependent increase in the antibacterial response was observed with Se-NPs against test organisms, while the same NPs caused inhibition at highest concentration against Gram negative *E. coli* (Richtera et al. 2015).

One-pot biosynthesis of four nanocomposites (NCs) by using TiO_2–Ag, Ag–TiO_2, Cu–Ag, and Ag–Cu combination was reported, and the NCs were tested for their possible antiquorum sensing, antiplanktonic, antiswarming motility, and antibiofilm activities against MDR strains. Ag–TiO_2 NCs showed strong evidence of decrease in the biofilm roughness, pyocyanin synthesis, and lowering of swarming motility of *P. aeruginosa* than control samples. Antibiofilm strength for these NCs were in the order of Ag–TiO_2>TiO_2–Ag>Cu–Ag>Ag–Cu. Agar diffusion method was used to determine MIC and MBC (Alavi and Karimi 2018).

Au-nanoparticles exhibiting smaller geometry, well-developed surface chemistry, chemical stability, and structural rigidity offer as an ideal tool to study the effects of conjugation on its properties. In a study, authors selected vancomycin as an antibiotic

to load on to Au-nanoparticles (AuNPs) and evaluated their activity against vancomycin-resistant enterococci (VRE). They devised a chemical route to synthesize Au-Van nanoparticles, which showed significantly improved activity against vancomycin-resistant *E. faecium* and *E. coli* (Gu et al. 2003).

In a recent study authors demonstrated synergistic effect of silver-minocycline combination. In the study, a metabolite of minocycline, 4-epi-minocycline, was detected as an active antimicrobial against resistant *P. aeruginosa* by use of a high-throughput screen. Nanoparticles loaded with silver and minocycline were tested against *P. aeruginosa* clinical isolates. Minocycline and silver when used alone were very effective while the combination was superior to individual entities, allowing reduction in dose for the both therapeutics to obtain similar antimicrobial effect (Chen et al. 2019).

In another study, authors proposed use of biocompatible nanomedicines as an alternative to existing antibiotics in which a novel antibacterial system against MDR strains was fabricated. They made use of modified versions of naturally occurring antimicrobial peptides in conjugation with Ag-NPs to create these novel materials. For this, a cysteine residue was introduced either at one of the terminal of the parent peptide as a strategy to enhance binding and antimicrobial potential of the resultant peptide with the AgNPs. The cysteine-tagged NCs showed MIC values of 5–15 μM as compared to 50 μM for peptides devoid of the cysteine residues. NMR spectroscopy and molecular simulations revealed a hydrophobic collapse mechanism triggering pore formation in the bilayer membrane for the improved antibacterial effects of cysteine-tagged NCs. The microbial strains chosen for the study were *K. pneumoniae*, *P. aeruginosa*, and *S. typhi* (Pal et al. 2019). Similar advantages of nanoparticle tagging and as a function of size and shape were studied by Liu et al. (2013) where the importance of cysteine in the conjugation process was demonstrated (Liu et al. 2013).

4.5 Metal Nanoparticles & Multidrug-Resistant Model Strains

Many types of metal NPs, metal oxide nanoparticles and their conjugates are being developed and evaluated against multidrug-resistant pathogens (MDRs) for their antimicrobial potential. In some cases, metal nanoparticles not only showed activity against the MDR strains but also displayed synergy by carrying and delivering cargo such as antibiotics or other natural antimicrobials (Linlin et al. 2017).

In a study, ZnO-NPs of 15 nm size were obtained through biogenic route by use of leaf extract *of Aloe barbadensis* Miller *(A. vera*). Through surface binding and subsequent internalization, the synthesized ZnO-NPs inhibited *S. aureus* and *E. coli*. Significant antibacterial and anti-biofilm potential were also detected against clinical isolates of MRSA and extended spectrum beta-lactamases (ESBL) positive *E. coli, P. aeruginosa*, with the MIC and MBC values of 2400, 2200 μg/ml and 2700, 2300 μg/ml, respectively (Ali et al. 2016).

Two carbapenems (a β-lactam antibiotic)- imipenem (Ipm) and meropenem (Mem) were individually conjugated to surface of Au-nanoparticles by citrate reduction method to maximize its therapeutic antibacterial potential against

drug-resistant pathogens. The nanoparticles of 35 nm size each thus obtained showed maximum activity against carbapenem-resistant Gram-negative clinical isolates - *K. pneumoniae, Proteus mirabilis* and *Acinteobacter baumanii*. A four-fold decrease in MIC of Ipm and a three-fold decrease in MIC of Mem were observed (Shaker and Shaaban 2017).

Gold nanoparticles prepared with the help of chicken egg white protein (CEW) as reducing and stabilizing agent were further coated with 2-mercapto-1-methylimidazole (MMT) molecules. The resultant Au-CEW-MMT nanoparticles were highly active against MDR pathogens. Further in vivo study using a rabbit model proved the nanocomposite to have effective wound healing properties (Lu et al. 2018).

In an unconventional approach, a synergistic effect was observed when antibiotic-tagged gold nanoparticles (AuNPs) and a targeted pulsed laser therapy enhanced antibiotic efficacy against MDR strains. AuNPs- in combination with gentamicin or amikacin antibiotics and targeted pulsed laser therapy caused a significant 4 to 5-log reduction in the viability of methicillin-resistant *S. aureus* and *P. aeruginosa* biofilms, respectively, compared to ~1 log reduction of when treatments were used alone (Kirui et al. 2019).

Spherical silver nanoparticles of 20–50 nm dimensions were synthesized (AgNPs) through a rapid, single pot bio-reduction method making use of *Nocardiopsis* sp. GRG1 (KT235640) biomass. The synthesized AgNPs showed antibacterial and anti-biofilm activities against a clinical isolate *Staphylococci,* at 5–60 µg/mL (Rajivgandhi et al. 2019a).

Silver nanoparticles produced using marine *Streptomyces* sp. Al-Dhabi-89 showed activity against *E.coli, P. aeruginosa*, *K. pneumoniae* and clinical drug-resistant microbial isolates MRSA and *P. mirabilis*. The NPs thus produced had cubic dimensions with 11–21 nm size range (Al-Dhabi et al. 2018).

Chang et al., reported use of glucose and trimethyl chitosan nitrate as reducing and stabilizing agent for the biosynthesis of trimethyl chitosan nitrate-capped silver nanoparticles (TMCN-AgNPs). The TMCN-AgNPs possessed antibacterial activity against *P. aeruginosa*, *E. coli*, and *S. aureus* at a concentration lower than 6.13 µg/mL and showed MIC of 12.25 µg/mL against multidrug-resistant *A. baumannii* strain (Chang et al. 2017).

Bacterial exopolysaccharide stabilized AgNPs were synthesized of 2–15 nm size range and were tested to be active against multidrug-resistant pathogens *P. aeruginosa* and *K. pneumoniae* (Kanmani and Lim 2013). In another study, biosynthesis of AgNPs was carried out using leaf extract of *Mukia scabrella* to obtain nanoparticles of 18–21 nm size with spherical shape. MDR- strains of *Acinetobacter* sp., *K. pneumoniae*, and *P. aeruginosa* were found to be sensitive in presence of these AgNPs (Prabakar et al. 2013).

A study reported synergistic action of chitosan along with zinc oxide nanomicelles (CZnO-NPs) against MDR strain for their complete elimination of test pathogens such as *E. coli* BAA-2471 and *E. faecium* 1449 (Bui et al. 2017).

Mehta et al. (2019) performed mechanistic studies using LIVE/DEAD viability assay, fluorescence imaging, 3D confocal microscopy and cytotoxicity assay to determine effective dose (ED50) of the miceller CZnO-NPs for elimination of the

MDR *E. faecium* 1449 as model. The results revealed that within 24 h the CZnONPs achieved 50.22% biofilm reduction compared to 15.66% with chitosan and 13.94% of ZnO alone. The chitosan coated ZnO-NPs (CZnONPs) showed promising action on MDR bacterial biofilms. The synergistic action has been attributed to the chitosan shell of CZNPs for controlled release of ZnO in the bacterial cell, whereby ZnO showed antibacterial property against Gram-positive species and also to chitosan's inherent antimicrobial activity mainly against Gram negative strains (Mehta et al. 2019).

Authors in a recent study showed for the first time pectin-capped biogenic platinum NPs (PtNPs) to cause selective loss of plasmid from capsulated strain of *E. coli* U3790 strain. These pectin-coated PtNPs acted as plasmid curing agent and showed a significant decline in MIC of 16-64 fold for meropenem and ceftriaxone in carbapenem-esistant *Escherichia coli* (CREC). Both in vitro and in vivo the plasmid cured strain showed smaller colonies and slower growth. It also lead to drastic reduction in bacterial bioburden by 2.4 log CFU compared to meropenem treatment alone. Acquisition of plasmid from wild-type strain into cured strain restablished drug-resistant phenotype. Mechanistic studies revealed that nanoparticles did not induce ROS formation but interacted with cell surface and perturbed inner membrane integrity (Bharathan et al. 2019).

In another study, nano-antibiotics (AgNAs) were prepared by the self-assembly of ultra-small silver-nanoclusters and biofilm-responsive polymeric ligands. The resultant AgNAs were responsive to the acidic conditions prevalent in the biofilm which caused ligand protonation and later their disaggregation. This feature of the AgNAs allowed enhanced retention, better penetration, and accelerated leaching of silver ions thus effectively killing the bacteria inside the biofilm, in comparison to regular antimicrobial agents which show limited permeation and therapeutic activity. These effects were confirmed on a methicillin-resistant *Staphylococcus aureus* (MRSA) infection model both in vitro and later in vivo which significantly reduced mortality rate of mice with biofilm-induced severe pyomyositis (Wu et al. 2019).

Authors in their study combined the antimicrobial effects of silver ion with selective toxicity of branched polyethylenimine (bPEI) to effectively kill MDR pathogenic strains by fabricating branched polyethylenimine-functionalized silver nanoclusters (bPEI-AgNCs). The MIC of bPEI-AgNCs was 10–15 folds lesser than that of PEI alone and 2–3 fold lower than $AgNO_3$ alone when determined against uropathogenic MDR strains (Huma et al. 2018).

In another study, glutathione-stabilized silver nanoparticles (GSH-AgNPs) were synthesized and their activity was evaluated against multidrug-resistant *Campylobacter* strains isolated from the chicken and patients. The MIC and minimal bactericidal concentration (MBC) were found to be 4.92–39.4 μg/mL and 9.85–39.4 μg/mL, respectively and the GSH-AgNPs remarkable activity against all MDR *Campylobacter* strains tested (Silvan et al. 2018).

ZnO nanoparticles (ZnO-NPs) of 30 nm dimension were synthesized and evaluated using dilution and disk diffusion assays to confirm their antimicrobial potential against carbapenem-resistant *A. baumannii*. The resultant ZnO-NPs demonstrated good antibacterial activity against the strain selected. Production of ROS triggering

leakage of cellular contents was proposed as the mechanism of reduction in cell viability in the pathogen (Tiwari et al. 2018). In another study, ZnO-NPs of 70 nm size with spherical or rod-shapes were prepared and tested at different concentrations as antimicrobial agent against a notorious foodborne pathogen *E. coli* O157:H7. The microbial growth inhibited at the MIC of 12 mmol/L. ZnO- NPs showed significant antimicrobial activity against *E. coli* O157:H7 by causing leakage of cellular contents and lysing the bacterial cells (Liu et al. 2009).

Bankier et al. (2018) engineered nanoparticle combinations (NPCs) by using silver-, copper nanoparticles (Ag-, Cu-NPs), and tungsten carbide (WC). Several literature is available on strong antimicrobial activity of NPCs against vast number of bacterial species. Different methods like plate count assay, viability assay by flow cytometry, and qPCR were used to evaluate the antimicrobial activity of prepared NPCs on *S. aureus* and *P. aeruginosa* bacteria at 0.05–0.25% concentration. The plate assay and flow cytometry confirmed the antimicrobial effects of NPCs to the extent of >8 log reduction (Bankier et al. 2018).

Ouyang et al. (2018) evaluated the use of black phosphorus (BP) together with AgNPs against methicillin-resistant *Staphylococcus aureus* (MRSA) model. Black phosphorus was used as a substrate, a stabilizing and a reducing agent and BP nanosheets were coated with AgNPs through an in situ growth method. Combination of near-infrared (NIR) light irradiation triggered the photothermal effect of the black phosphorus in Ag-BP nanohybrids and released Ag+ ions gradually to cause rapid disruption of bacterial membrane with enhanced the antibacterial effect. Further, the in vivo studies in mice model proved Ag-BP nanohybrids to be efficient in decreasing the MRSA bio-burden and thus reduce infection-related tissue lesions (Ouyang et al. 2018). The recent results of use of nanoparticles and nanomaterials against multidrug-resistant bacteria have been reviewed in detail by (Baptista et al. 2018). The use of some NPs targeting against MDR efflux pumps have been recently reviewed by Hasani et al. (2019) (Hasani et al. 2019). The Table 4.2 summarizes few examples of metal and metal oxide NPs against MDR strains as models.

4.6 Metal Nanoparticles and Plausible Toxicity or Resistance

Metal, metal oxides and other engineered forms of nanoparticles are an emerging class of antibacterials, finding their way to provide alternatives to antibiotics and also acting against resistant pathogens. Though, these materials are produced in large quantities globally, their impact on environment and life-forms warrants detailed evaluation studies in this direction. A study was carried out on various metal and metal oxide particles (TiO_2, Cu, CuO, Zn, ZnO, Fe_2O_4, Fe_3O_4, Fe_2O_3), and the toxicity was evaluated to that of multiwalled carbon nanotubes and carbon nanoparticles with the use of human lung epithelial cell line A549. The CuO-NPs were labeled to be most toxic and could cause severe DNA damage along with oxidative lesions. The toxicity was further pronounced in the presence of ZnO and TiO_2

Table 4.2 Representative examples of metal/ metal oxide NPs against MDR strains

Metal/Metal oxide NPs	Method of synthesis	Size	Shape	MDR Model organisms	Antibacterial mechanism	Reference
AgNPs	Single-pot biological reduction method- *Nocardiopsis* sp. GRG1 (KT235640) biomass	20 and 50 nm	Spherical	Positive methicillin-resistant coagulase-negative *Staphylococci*	Cellular damage	Rajivgandhi et al. (2019a)
Ag	Green synthesis- trimethyl chitosan nitrate-capped silver nanoparticles (TMCN-AgNPs)	81.2 nm	–	Gram-positive, Gram-negative, and *A. baumannii* MDR strains	Cellular damage	Chang et al. (2017)
AgNPs	Biosynthesis- silver tolerant mediated by bacteria (AgTB)	17.51 nm	Spherical	ESKAPE pathogens	Cell damage	Khan et al. (2019)
AgNPs	Biosynthesis- extracts of *Citrus maxima* plant	~120–400 nm	–	*B. cereus* and MDR- *S. enteritidis*	Cell damage	Jha et al. (2017)
AgNPs	Green synthesis- aqueous leaf extract of *Corchorus capsularis* (CRCP)	Average size of 20.52 nm	Spherical	*P. aeruginosa*, *S. aureus*, isolates from postsurgical wound infections	Cell damage	(Prabakar et al. (2013)
ZnO	Green synthesis- *Aloe vera* extract	15 nm	Spherical	Extended spectrum beta lactamases (ESBL) positive *E. coli*, *P. aeruginosa*, and MRSA clinical isolates	Cell damage	Ali et al. (2016)
CuO	Green synthesis- *Camilla japonica* plant leaf extract.	~15–25 nm	Spherical	Extended-spectrum beta-lactamases	Damage to bacterial membrane	Rajivgandhi et al. (2019b)

particles. While no or low toxicity was observed for remaining NPs. The carbon-nanotubes showed cytotoxicity at the lowest tested dose (Karlsson et al. 2008).

Another study investigated TiO_2 nanoparticles-induced genotoxicity, inflammation and oxidative DNA damage in a mice model. The results indicated that the NPs induced genotoxicity in vivo thus raising concerns about health hazards due to TiO_2 NPs exposure (Trouiller et al. 2009). Moreover, there are some findings that reported development of bacterial resistance to metal NPs. For instance, a recent study provided insights on impact of NPs on spread of antibiotic resistance. The results indicated that both metal and its metal oxide- i.e., copper (Cu^{2+}) and copper oxide nanoparticles (CuO-NPs) were able to stimulate multiple-drug resistance genes via the conjugative transfer. At sub-inhibitory or environmental concentrations, the plasmid-mediated antibiotic resistance genes were found to be significantly upregulated (Zhang et al. 2019).

Such evidences are a matter of greater concern and need elaborate studies to quantify and postulate mechanisms of resistance and toxicity in future.

4.7 Conclusion

The recent scientific advances in the nanotechnology domain with special reference to metal/metal oxide nanoparticles and nanocomposites as antibacterial agents have opened up new opportunities to combat infections that are caused by bacterial pathogens. The findings summarized in present body of work as a function of - nature of metal, shape, size, concentration, and nature of conjugation, show promising results in support of these nanomaterials to act against microbial species and their multidrug-resistant variants. Likewise, some observations reported recently suggest plausible health hazard based on their capability to cross cellular barriers and risk of development of resistance among microbial pathogens against metal NPs. As some of these nanomaterials are already in the consumer market, for other diverse applications, the future research and actions should be directed towards systematic evaluation of its benefits and its effects on human health. It thus demands caution to be exercised before the large-scale exposure of these wonder materials in an environmental sphere and food-chain continuation of nature.

References

Al-Dhabi NA, Ghilan AKM, Arasu MV, Duraipandiyan V (2018) Green biosynthesis of silver nanoparticles produced from marine *Streptomyces* sp. Al-Dhabi-89 and their potential applications against wound infection and drug resistant clinical pathogens. J Photochem Photobiol B Biol 189:176–184. https://doi.org/10.1016/j.jphotobiol.2018.09.012

Al-Hazmi F, Alnowaiser F, Al-Ghamdi AA, Al-Ghamdi AA, Aly MM, Al-Tuwirqi RM, El-Tantawy F (2012) A new large-scale synthesis of magnesium oxide nanowires: structural and antibacterial properties. Superlattice Microstruct 52:200–209. https://doi.org/10.1016/j.spmi.2012.04.013

Alavi M, Karimi N (2018) Antiplanktonic, antibiofilm, antiswarming motility and antiquorum sensing activities of green synthesized Ag–TiO_2, TiO_2–Ag, Ag–Cu and Cu–Ag nanocomposites against multi-drug-resistant bacteria. Artif Cells Nanomed Biotechnol 46:S399–S413. https://doi.org/10.1080/21691401.2018.1496923

Ali K, Dwivedi S, Azam A, Saquib Q, Al-Said MS, Alkhedhairy AA, Musarrat J (2016) Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates. J Colloid Interface Sci 472:145–156. https://doi.org/10.1016/j.jcis.2016.03.021

Arakha M, Pal S, Samantarrai D, Panigrahi TK, Mallick BC, Pramanik K, Mallick B, Jha S (2015) Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. Sci Rep 5:1–12. https://doi.org/10.1038/srep14813

Azam A, Ahmed AS, Oves M, Khan MS, Memic A (2012) Size-dependent antimicrobial properties of CuO nanoparticles against gram-positive and -negative bacterial strains. Int J Nanomedicine 7:3527–3535. https://doi.org/10.2147/IJN.S29020

Bankier C, Cheong Y, Mahalingam S, Edirisinghe M, Ren G, Cloutman-Green E, Ciric L (2018) A comparison of methods to assess the antimicrobial activity of nanoparticle combinations on bacterial cells. PLoS One 13:1–13. https://doi.org/10.1371/journal.pone.0192093

Baptista PV, McCusker MP, Carvalho A, Ferreira DA, Mohan NM, Martins M, Fernandes AR (2018) Nano-strategies to fight multidrug resistant bacteria-"a Battle of the titans". Front Microbiol 9:1–26. https://doi.org/10.3389/fmicb.2018.01441

Baranwal A, Srivastava A, Kumar P, Bajpai VK, Maurya PK, Chandra P (2018) Prospects of nanostructure materials and their composites as antimicrobial agents. Front Microbiol 9:422. https://doi.org/10.3389/fmicb.2018.00422

Bharathan S, Sundaramoorthy NS, Chandrasekaran H, Rangappa G, ArunKumar G, Subramaniyan SB, Veerappan A, Nagarajan S (2019) Sub lethal levels of platinum nanoparticle cures plasmid and in combination with carbapenem, curtails carbapenem resistant *Escherichia coli*. Sci Rep 9:1–13. https://doi.org/10.1038/s41598-019-41489-3

Bhardwaj N, Pandey SK, Mehta J, Bhardwaj SK, Kim KH, Deep A (2018) Bioactive nano-metal-organic frameworks as antimicrobials against gram-positive and gram-negative bacteria. Toxicol Res (Camb) 7:931–941. https://doi.org/10.1039/c8tx00087e

Bogdanović U, Lazić V, Vodnik V, Budimir M, Marković Z, Dimitrijević S (2014) Copper nanoparticles with high antimicrobial activity. Mater Lett 128:75–78. https://doi.org/10.1016/j.matlet.2014.04.106

Bui VKH, Park D, Lee YC (2017) Chitosan combined with ZnO, TiO_2 and Ag nanoparticles for antimicrobialwound healing applications: a mini review of the research trends. Polymers (Basel) 9:21. https://doi.org/10.3390/polym9010021

Chang TY, Chen CC, Cheng KM, Chin CY, Chen YH, Chen XA, Sun JR, Young JJ, Chiueh TS (2017) Trimethyl chitosan-capped silver nanoparticles with positive surface charge: their catalytic activity and antibacterial spectrum including multidrug-resistant strains of *Acinetobacter baumannii*. Colloids Surf B Biointerfaces 155:61–70

Chatterjee AK, Chakraborty R, Basu T (2014) Mechanism of antibacterial activity of copper nanoparticles. Nanotechnology 25:135101. https://doi.org/10.1088/0957-4484/25/13/135101

Chen Q, Shah KN, Zhang F, Salazar AJ, Shah PN, Li R, Sacchettini JC, Wooley KL, Cannon CL (2019) Minocycline and silver dual-loaded polyphosphoester-based nanoparticles for treatment of resistant *Pseudomonas aeruginos*a. Mol Pharm 16:1606–1619. https://doi.org/10.1021/acs.molpharmaceut.8b01288

Cioffi N, Rai M (2012) Nano-antimicrobials: progress and prospects. Springer, Berlin

Duffy LL, Osmond-McLeod MJ, Judy J, King T (2018) Investigation into the antibacterial activity of silver, zinc oxide and copper oxide nanoparticles against poultry-relevant isolates of *Salmonella* and *Campylobacter*. Food Control 92:293–300. https://doi.org/10.1016/j.foodcont.2018.05.008

Foster HA, Ditta IB, Varghese S, Steele A (2011) Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity. Appl Microbiol Biotechnol 90:1847–1868. https://doi.org/10.1007/s00253-011-3213-7

Girase B, Depan D, Shah JS, Xu W, Misra RDK (2011) Silver-clay nanohybrid structure for effective and diffusion-controlled antimicrobial activity. Mater Sci Eng C 31:1759–1766. https://doi.org/10.1016/j.msec.2011.08.007

Gu H, Ho PL, Tong E, Wang L, Xu B (2003) Presenting vancomycin on nanoparticles to enhance antimicrobial activities. Nano Lett 3:1261–1263. https://doi.org/10.1021/nl034396z

Hamilton WL, Wenlock R (2016) Antimicrobial resistance: a major threat to public health. Cambridge Med J. https://doi.org/10.7244/cmj.2016.01.001

Hasani A, Madhi M, Gholizadeh P, Mojarrad JS, Rezaee MA, Zarrini G, Kafil HS (2019) Metal nanoparticles and consequences on multi-drug resistant bacteria: reviving their role. SN Appl Sci 1:1–13. https://doi.org/10.1007/s42452-019-0344-4

Hazan R, Beyth N, Khan W, Khan W, Hazan R (2015) Alternative antimicrobial approach: nano-antimicrobial materials. Evid Based Complement Alternat Med 2015:1–16. https://doi.org/10.1155/2015/246012

He Y, Ingudam S, Reed S, Gehring A, Strobaugh TP Jr, Irwin P (2016) Study on the mechanism of antibacterial action of magnesium oxide nanoparticles against foodborne pathogens. J Nanobiotechnol 14:1–9. https://doi.org/10.1186/s12951-016-0202-0

Huma ZE, Gupta A, Javed I, Das R, Hussain SZ, Mumtaz S, Hussain I, Rotello VM (2018) Cationic silver nanoclusters as potent antimicrobials against multidrug-resistant bacteria. ACS Omega 3:16721–16727. https://doi.org/10.1021/acsomega.8b02438

Jesline A, John NP, Narayanan PM et al (2015) Antimicrobial activity of zinc and titanium dioxide nanoparticles against biofilm-producing methicillin-resistant *Staphylococcus aureus*. Appl Nanosci 5:157–162. https://doi.org/10.1007/s13204-014-0301-x

Jeyaraj Pandian C, Palanivel R, Dhanasekaran S (2016) Screening antimicrobial activity of nickel nanoparticles synthesized using *Ocimum sanctum* leaf extract. J Nanopart 2016:1–13. https://doi.org/10.1155/2016/4694367

Jha D, Thiruveedula PK, Pathak R, Kumar B, Gautam HK, Agnihotri S, Sharma AK, Kumar P (2017) Multifunctional biosynthesized silver nanoparticles exhibiting excellent antimicrobial potential against multi-drug resistant microbes along with remarkable anticancerous properties. Mater Sci Eng C 80:659–669. https://doi.org/10.1016/j.msec.2017.07.011

Jin T, He Y (2011) Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. J Nanopart Res 13:6877–6885. https://doi.org/10.1007/s11051-011-0595-5

Jin T, Sun D, Su JY, Zhang H, Sue HJ (2009) Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogenes*, *Salmonella enteritidis*, and *Escherichia coli* O157:H7. J Food Sci 74:M46–M52. https://doi.org/10.1111/j.1750-3841.2008.01013.x

Kanmani P, Lim ST (2013) Synthesis and structural characterization of silver nanoparticles using bacterial exopolysaccharide and its antimicrobial activity against food and multidrug resistant pathogens. Process Biochem 48:1099–1106. https://doi.org/10.1016/j.procbio.2013.05.011

Karlsson HL, Cronholm P, Gustafsson J, Möller L (2008) Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chem Res Toxicol 21:1726–1732. https://doi.org/10.1021/tx800064j

Khalid HF, Tehseen B, Sarwar Y, Hussain SZ, Khan WS, Raza ZA, Bajwa SZ, Kanaras AG, Hussain I, Rehman A (2019) Biosurfactant coated silver and iron oxide nanoparticles with enhanced anti-biofilm and anti-adhesive properties. J Hazard Mater 364:441–448. https://doi.org/10.1016/j.jhazmat.2018.10.049

Khan MH, Unnikrishnan S, Ramalingam K (2019) Bactericidal potential of silver-tolerant bacteria derived silver nanoparticles against multi drug resistant ESKAPE pathogens. Biocatal Agric Biotechnol 18:100939. https://doi.org/10.1016/j.bcab.2018.12.004

Khan ST, Musarrat J, Al-Khedhairy AA (2016) Countering drug resistance, infectious diseases, and sepsis using metal and metal oxides nanoparticles: current status. Colloids Surf B Biointerfaces 146:70–83. https://doi.org/10.1016/j.colsurfb.2016.05.046

Kirui DK, Weber G, Talackine J, Millenbaugh NJ (2019) Targeted laser therapy synergistically enhances efficacy of antibiotics against multi-drug resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. Nanomed Nanotechnol Biol Med 20:102018. https://doi.org/10.1016/j.nano.2019.102018

Lallo da Silva B, Caetano BL, Chiari-Andréo BG et al (2019) Increased antibacterial activity of ZnO nanoparticles: influence of size and surface modification. Colloids Surf B Biointerfaces 177:440–447. https://doi.org/10.1016/j.colsurfb.2019.02.013

Lee JH, Kim YG, Cho MH, Lee J (2014) ZnO nanoparticles inhibit *Pseudomonas aeruginosa* biofilm formation and virulence factor production. Microbiol Res 169(12):888–896

Linlin W, Chen H, Longquan S (2017) 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

Liu L, Yang J, Xie J, Luo Z, Jiang J, Yang YY, Liu S (2013) The potent antimicrobial properties of cell penetrating peptide-conjugated silver nanoparticles with excellent selectivity for gram-positive bacteria over erythrocytes. Nanoscale 5:3834–3840. https://doi.org/10.1039/c3nr34254a

Liu Y, He L, Mustapha A, Li H, Hu ZQ, Lin M (2009) Antibacterial activities of zinc oxide nanoparticles against *Escherichia coli* O157:H7. J Appl Microbiol 107:1193–1201. https://doi.org/10.1111/j.1365-2672.2009.04303.x

Lu B, Lu F, Ran L, Yu K, Xiao Y, Li Z, Dai F, Wu D, Lan G (2018) Self-assembly of natural protein and imidazole molecules on gold nanoparticles: applications in wound healing against multi-drug resistant bacteria. Int J Biol Macromol 119:505–516. https://doi.org/10.1016/j.ijbiomac.2018.07.167

Lungu M, Gavriliu Ş, Enescu E, Ion L, Brătulescu A, Mihăescu G, Măruţescu L, Chifiriuc MC (2014) Silver-titanium dioxide nanocomposites as effective antimicrobial and antibiofilm agents. J Nanopart Res 16:2203. https://doi.org/10.1007/s11051-013-2203-3

Marega C, Maculan J, Andrea Rizzi G, Saini R, Cavaliere E, Gavioli L, Cattelan M, Giallongo G, Marigo A, Granozzi G (2015) Polyvinyl alcohol electrospun nanofibers containing Ag nanoparticles used as sensors for the detection of biogenic amines. Nanotechnology 26:75501. https://doi.org/10.1088/0957-4484/26/7/075501

Martinez-Gutierrez F, Olive PL, Banuelos A, Orrantia E, Nino N, Sanchez EM, Ruiz F, Bach H, Av-Gay Y (2010) Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. Nanomed Nanotechnol Biol Med 6:681–688. https://doi.org/10.1016/j.nano.2010.02.001

Mehta M, Allen-Gipson D, Mohapatra S, Kindy M, Limayem A (2019) Study on the therapeutic index and synergistic effect of chitosan-zinc oxide nanomicellar composites for drug-resistant bacterial biofilm inhibition. Int J Pharm 565:472–480. https://doi.org/10.1016/j.ijpharm.2019.05.003

Mohan Kumar K, Mandal BK, Appala Naidu E, Sinha M, Siva Kumar K, Sreedhara Reddy P (2013) Synthesis and characterisation of flower shaped zinc oxide nanostructures and its antimicrobial activity. Spectrochim Acta A Mol Biomol Spectrosc 104:171–174. https://doi.org/10.1016/j.saa.2012.11.025

Mosaiab T, Jeong CJ, Shin GJ, Choi KH, Lee SK, Lee I, In I, Park SY (2013) Recyclable and stable silver deposited magnetic nanoparticles with poly(vinyl pyrrolidone)-catechol coated iron oxide for antimicrobial activity. Mater Sci Eng C 33:3786–3794. https://doi.org/10.1016/j.msec.2013.05.009

Mukherjee A, Mohammed Sadiq I, Prathna TC, Chandrasekaran N (2011) Antimicrobial activity of aluminium oxide nanoparticles for potential clinical applications. In: Science against microbial pathogens: communicating current research and technological advances. Formatex Research Center, Badajoz, Spain, pp 245–251

Muthukrishnan L, Chellappa M, Nanda A (2019) Bio-engineering and cellular imaging of silver nanoparticles as weaponry against multidrug resistant human pathogens. J Photochem Photobiol B Biol 194:119–127. https://doi.org/10.1016/j.jphotobiol.2019.03.021

Nejabatdoust A, Zamani H, Salehzadeh A (2019) Functionalization of ZnO nanoparticles by glutamic acid and conjugation with thiosemicarbazide alters expression of efflux pump genes in multiple drug-resistant *Staphylococcus aureus* strains. Microb Drug Resist 25:966–974. https://doi.org/10.1089/mdr.2018.0304

Nombona N, Antunes E, Chidawanyika W, Kleyi P, Tshentu Z, Nyokong T (2012) Synthesis, photophysics and photochemistry of phthalocyanine-ε-polylysine conjugates in the presence of metal nanoparticles against *Staphylococcus aureus*. J Photochem Photobiol A Chem 233:24–33. https://doi.org/10.1016/j.jphotochem.2012.02.012

Otari SV, Patil RM, Nadaf NH, Ghosh SJ, Pawar SH (2014) Green synthesis of silver nanoparticles by microorganism using organic pollutant: its antimicrobial and catalytic application. Environ Sci Pollut Res 21:1503–1513. https://doi.org/10.1007/s11356-013-1764-0

Ouyang J, Liu RY, Chen W, Liu Z, Xu Q, Zeng K, Deng L, Shen L, Liu Y-N (2018) A black phosphorus based synergistic antibacterial platform against drug resistant bacteria. J Mater Chem B 6:6302–6310. https://doi.org/10.1039/c8tb01669k

Pal I, Bhattacharyya D, Kar RK, Zarena D, Bhunia A, Atreya HS (2019) A peptide-nanoparticle system with improved efficacy against multidrug resistant bacteria. Sci Rep 9:1–11. https://doi.org/10.1038/s41598-019-41005-7

Palza H, Delgado K, Curotto N (2015) Synthesis of copper nanostructures on silica-based particles for antimicrobial organic coatings. Appl Surf Sci 357:86–90. https://doi.org/10.1016/j.apsusc.2015.08.260

Pazos-Ortiz E, Roque-Ruiz JH, Hinojos-Márquez EA, López-Esparza J, Donohué-Cornejo A, Cuevas-González JC, Espinosa-Cristóbal LF, Reyes-López SY (2017) Dose-dependent antimicrobial activity of silver nanoparticles on polycaprolactone fibers against gram-positive and gram-negative bacteria. J Nanomater 2017:1–9. https://doi.org/10.1155/2017/4752314

Prabakar K, Sivalingam P, Mohamed Rabeek SI, Muthuselvam M, Devarajan N, Arjunan A, Karthick R, Suresh MM, Wembonyama JP (2013) Evaluation of antibacterial efficacy of phyto fabricated silver nanoparticles using *Mukia scabrella* (Musumusukkai) against drug resistance nosocomial gram negative bacterial pathogens. Colloids Surf B Biointerfaces 104:282–288. https://doi.org/10.1016/j.colsurfb.2012.11.041

Prasad R, Pandey R, Barman I (2016) Engineering tailored nanoparticles with microbes: quo vadis? Wiley Interdiscip Rev Nanomed Nanobiotechnol 8:316–330. https://doi.org/10.1002/wnan.1363

Premanathan M, Karthikeyan K, Jeyasubramanian K, Manivannan G (2011) Selective toxicity of ZnO nanoparticles toward gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. Nanomedicine Nanotechnology, Biol Med 7:184–192. https://doi.org/10.1016/j.nano.2010.10.001

Prucek R, Tuček J, Kilianová M, Panáček A, Kvítek L, Filip J, Kolář M, Tománková K, Zbořil R (2011) The targeted antibacterial and antifungal properties of magnetic nanocomposite of iron oxide and silver nanoparticles. Biomaterials 32:4704–4713. https://doi.org/10.1016/j.biomaterials.2011.03.039

Rajivgandhi G, Maruthupandy M, Muneeswaran T, Anand M, Quero F, Manoharan N, Li W-J (2019a) Biosynthesized silver nanoparticles for inhibition of antibacterial resistance and biofilm formation of methicillin-resistant coagulase negative *Staphylococci*. Bioorg Chem 89:103008. https://doi.org/10.1016/j.bioorg.2019.103008

Rajivgandhi G, Maruthupandy M, Muneeswaran T, Ramachandran G, Manoharan N, Quero F, Anand M, Song JM (2019b) Biologically synthesized copper oxide nanoparticles enhanced intracellular damage in ciprofloxacin resistant ESBL producing bacteria. Microb Pathog 127:267–276. https://doi.org/10.1016/j.micpath.2018.12.017

Razeeb KM, Podporska-Carroll J, Jamal M, Hasan M, Nolan ME, McCormack DE, Quilty B, Newcomb SB, Pillai SC (2014) Antimicrobial properties of vertically aligned nano-tubular copper. Mater Lett 128:60–63. https://doi.org/10.1016/j.matlet.2014.04.130

Richtera L, Chudobova D, Cihalova K, Kremplova M, Milosavljevic V, Kopel P, Blazkova I, Hynek D, Adam V, Kizek R (2015) The composites of graphene oxide with metal or semimetal nanoparticles and their effect on pathogenic microorganism. Materials (Basel) 8:2994–3011. https://doi.org/10.3390/ma8062994

Rodrigues AG, Romano de Oliveira Gonçalves PJ, Ottoni CA, de Cássia Ruiz R, Morgano MA, de Araújo WL, de Melo IS, De Souza AO (2019) Functional textiles impregnated with bio-

genic silver nanoparticles from *Bionectria ochroleuca* and its antimicrobial activity. Biomed Microdevices 21:56. https://doi.org/10.1007/s10544-019-0410-0

Rudramurthy GR, Swamy MK, Sinniah UR, Ghasemzadeh A (2016) Nanoparticles: alternatives against drug-resistant pathogenic microbes. Molecules 21:1–30. https://doi.org/10.3390/molecules21070836

Sánchez-Sanhueza G, Fuentes-Rodríguez D, Bello-Toledo H (2016) Copper nanoparticles as potential antimicrobial agent in disinfecting root canals: a systematic review. Int J Odontostomatol 10:547–554. https://doi.org/10.4067/s0718-381x2016000300024

Schwegmann H, Frimmel FH (2010) Nanoparticles: interaction with microorganisms. In: Frimmel FH, Niessner R (eds) Nanoparticles in the water cycle. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 165–182

Seil JT, Webster TJ (2012) Antimicrobial applications of nanotechnology: methods and literature. Int J Nanomedicine 7:2767–2781. https://doi.org/10.2147/IJN.S24805

Shaker MA, Shaaban MI (2017) Formulation of carbapenems loaded gold nanoparticles to combat multi-antibiotic bacterial resistance: *In vitro* antibacterial study. Int J Pharm 525:71–84. https://doi.org/10.1016/j.ijpharm.2017.04.019

Silvan JM, Zorraquin-Peña I, de Llano DG, Moreno-Arribas MV, Martinez-Rodriguez AJ (2018) Antibacterial activity of glutathione-stabilized silver nanoparticles against *Campylobacter* multidrug-resistant strains. Front Microbiol 9:1–10. https://doi.org/10.3389/fmicb.2018.00458

Singh S, Bahadur D (2019) Highly efficient and reusable dendritic Fe3O4 magnetic Nanoadsorbent for inhibition of bacterial growth. Surfaces and Interfaces:100348. https://doi.org/10.1016/j.surfin.2019.100348

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

Sudhakar K, Moloi SJ, Madhusudhana Rao K (2017) Green synthesis and characterization of Halloysite Nanoclay/Curcumin/Ag hybrid Nano materials for antibacterial applications. J Inorg Organomet Polym Mater 27:1450–1456. https://doi.org/10.1007/s10904-017-0600-2

Tang J, Chen Q, Xu L et al (2013) Graphene oxide-silver nanocomposite as a highly effective antibacterial agent with species-specific mechanisms. ACS Appl Mater Interfaces 5:3867–3874. https://doi.org/10.1021/am4005495

Tiwari V, Mishra N, Gadani K et al (2018) Mechanism of anti-bacterial activity of zinc oxide nanoparticle against Carbapenem-resistant *Acinetobacter baumannii*. Front Microbiol 9:1–10. https://doi.org/10.3389/fmicb.2018.01218

Trouiller B, Reliene R, Westbrook A et al (2009) Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. Cancer Res 69:8784–8789. https://doi.org/10.1158/0008-5472.CAN-09-2496

Tsuang YH, Sun JS, Huang YC et al (2008) Studies of photokilling of bacteria using titanium dioxide nanoparticles. Artif Organs 32:167–174. https://doi.org/10.1111/j.1525-1594.2007.00530.x

Willems NIT (2005) Roadmap report on nanoparticles. Methodology:1–57

Wu J, Li F, Hu X et al (2019) Responsive assembly of silver Nanoclusters with a biofilm locally amplified bactericidal effect to enhance treatments against multi-drug-resistant bacterial infections. ACS Cent Sci 5:1366–1376. https://doi.org/10.1021/acscentsci.9b00359

Zare Y, Shabani I (2016) Polymer/metal nanocomposites for biomedical applications. Mater Sci Eng C 60:195–203. https://doi.org/10.1016/j.msec.2015.11.023

Zhang S, Wang Y, Song H et al (2019) Copper nanoparticles and copper ions promote horizontal transfer of plasmid-mediated multi-antibiotic resistance genes across bacterial genera. Environ Int 129:478–487. https://doi.org/10.1016/j.envint.2019.05.054