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Review

Antibacterial Properties of Metal and Metal Oxide Nanoparticles-An Overview

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Abstract: Nanotechnology has made a tremendous impact over a period of three decades in various facets of industrial and medical sectors and still find recent interests to exploit their characteristics for commercial applications. Nanobiotechnology is a field of using nanomaterials for biological applications. The preparation of nanomaterials from biological sources has been known as 'green' technology. Nanoparticles measure in the size range of 1-100 nm and possess unique characteristics than their bulk materials with potential applications in various biological, medical, industrial, agricultural and ecological fields. Using nanoparticles in antimicrobial applications has been explored since the early years of Nanotechnology development. However, few reports exist on the mechanisms of antimicrobial actions mediated by nanoparticles and their effects on environment and ecosystems. Several factors affect the antimicrobial properties of nanoparticles, which are quite important to understand the roles of their antimicrobial action. This review is aimed to discuss the antibacterial properties, the mechanisms and antimicrobial applications of Nanoparticles in medicine and allied areas. We have suggested that despite the several applications of nanomaterials, their production by biological methods and suitable applications also need to focus on the toxicity implications. Nanomaterial toxicity in the ecological niche is of significant concern and regulatory bodies should be organized to evaluate the successful use of nanomaterials for a wide variety of applications. The review significantly summarizes hot topics of interest in antibacterial activities, mode of action, biomedical applications of nanoparticles, their metabolic fate in vivo and toxicity effects in humans for further consideration of researches in Microbial Nanotechnology.

***Keywords*:** antimicrobial mechanisms, ecosystem, in vivo metabolism, metal oxide nanoparticles, nanomaterial toxicity

1. Introduction

Antibiotics are natural or synthetic compounds, which slow down or kill bacterial growth without toxicity to the surrounding tissues. Most antibiotics are natural compounds that can be chemically modified while some are

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synthesized commercially. Antibiotics which kill bacteria are bactericidal and which slow down bacterial growth are bacteriostatic [1-2]. Antibiotics are important in treating infectious diseases and are effective, easily available and affordable. Although effective alternatives to antibiotics are not known till date, improper use of antibiotics causes bacterial resistance leading to chronic infections and development of Multidrug-Resistance (MDR) bacterial strains [3]. During antibiotic therapy, resistant bacteria carrying a super-resistance gene NDM-1 may lead to genetically inheritable drug resistance based on evolutionary processes [4]. These antibiotic resistant bacteria commonly called as superbugs exhibit resistance to many antibiotics that were treated previously. Every year, multidrug-resistant tuberculosis (MDR-TB) occurs in about half a million populations worldwide [5]. Administration of high-doses of antibiotics is required for infections with drug resistance, which generate toxic and adverse side effects. Hence, the development and search for alternative strategies are deemed necessary to treat multi-drug resistance [6-8].

Up to date, antibiotics act on three major bacterial targets: the cell wall synthesis, translational machinery, and DNA replication machinery. Bacteria have developed resistance for all of these mechanisms. They produce enzymes that modify or degrade antibiotics, such as β-lactamases and aminoglycosidases [9]; modify cell wall and ribosomes against vancomycin and tetracycline respectively [10] and prevent the entry of antibiotics via efflux pumps [11].

Recently, metallic nanoparticles (NPs) are attractting attention from research community worldwide due to their antibacterial properties. These NPs are in the dimensions of 1-100 nm and are used to treat infections by targeting the delivery of antibiotics. Nanoparticles have proven effectiveness in their actions against infectious diseases caused by antibiotic resistant organisms in both *in vitro* and *in vivo* [12-13]. NPs are broad-spectrum towards both Gram-positive and Gram-negative bacteria. The use of metal NPs to treat antibiotic resistance meets all the significant criteria as antimicrobial drugs. Common metal NPs include Ag, Au, Cu, Zn and their oxides in a range of shapes and sizes in the nano regime [14]. Metal and Metal oxide NPs effectively treat multidrug resistance bacterial infections in many studies. ZnO NPs are effective against *Staphylococcus aureus*, while, Ag NPs inhibit *Escherichia coli* and *Pseudomonas aeruginosa* in a concentration-dependent manner [15]. However, the antibacterial mechanisms of NPs have still not been understood and the same NPs can exhibit contrasting activities towards similar bacteria. The antimicrobial mechanism of action of NPs can be via: oxidative stress induction [16], Metal ion release [17], or non-oxidative mechanisms [18] either simultaneously or individually. Ag NPs exert neutralization of the surface charge of the bacterial cell with changes in membrane permeability and eventually leads to cell death [19]. This review gives overall insights of the mechanisms of antibacterial resistance, antibacterial mechanisms of NPs, medical uses of NPs and other promising applications of NPs.

2. Mechanisms of antibacterial resistance

Antibacterial resistance causes a serious problem due to the indiscriminate use of antibiotics, for prophylactic treatment without appropriate medical advice. The improper use and choice of antimicrobials and continual changes in antimicrobial treatments lead to antimicrobial resistance. The indiscriminate use of antibiotics in poultry and animal husbandry and other sectors are some of the main causes in the development of resistance bacteria. Bacterial infections, which are treated with the administration of high dosage of an antibiotic [12], and use of multiple antibiotic combinations [20-21] also contribute to the development of multidrug-resistant strains. The following are the main mechanisms of antimicrobial resistance (Figure 1).

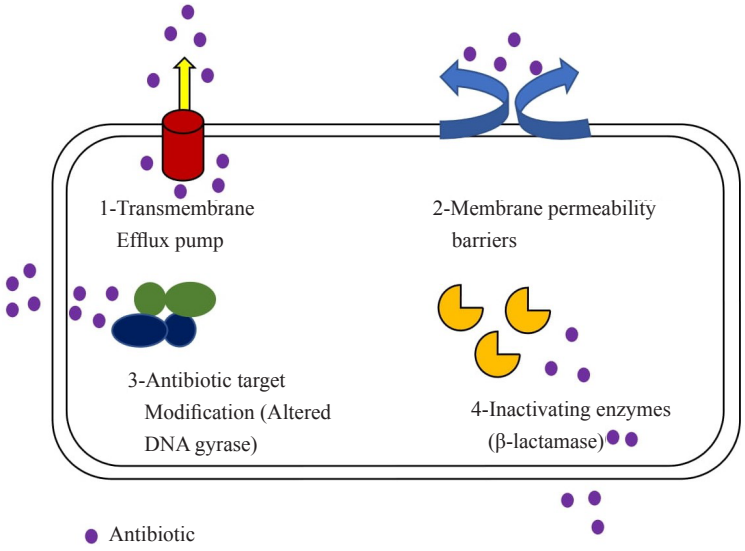

Figure 1. Mechanisms of Antibiotic Resistance in bacteria

2.1 *At the genetic level*

Depending on the source of resistance genes, antibiotic resistance may be either intrinsic or acquired. Intrinsic resistance is due to spontaneous mutations of existing genes, whereas acquired resistance is by the acquisition of genes from other resistant bacterial species. Multidrug resistance usually occurs by gaining different drug resistance genes by the bacteria [22]. Generally, acquired resistance is of considerable importance than intrinsic resistance because resistance genes are mostly acquired. The resistance is transferred between bacteria by transposons [23], plasmids [24-25], and integrons [26].

2.2 *At the protein level*

Some drug resistance mechanisms are due to changes in structural and transporter proteins, enzymes and receptors on the cell membrane. These mechanisms are due to 1) active efflux pump systems [27-28]; 2) modification of targets; 3) generation of inactive enzymes; 4) antibiotic impermeability; 5) formation of biofilms [29]; 6) elimination of *Kat*G [30] or *Bam*A [31] proteins that can cause infection; 7) induction of an antagonist by changes in metabolic pathways; 8) increase in antibiotic inhibitors [32].

3. Antibacterial mechanisms of NPs

In the recent years, many researchers focus on potential antibacterial mechanisms of NPs due to their multifold applications in various medical fields [19]. Silver NPs can penetrate into biofilms by inhibiting the expression of genes [33]. Further, metallic NPs have the inherent capacity to alter bacterial metabolic activity [34], and eliminate resistant bacteria. The NPs establish contact with bacterial cells for their antibacterial function through van der Waals forces [35], electrostatic attraction [36], receptor-ligand [37] and hydrophobic interactions [38]. Upon contact with bacterial cells, NPs cross the cell membrane and influence the shape and function of the cell membrane and intracellular organelles as they gather along the metabolic pathway [39-41]. The proposed mechanisms of antibiotic resistance are: oxidative stress [16], metal ion release [42] and non-oxidative mechanisms [18]. The mechanisms of antibiotic resistance mediated by silver NPs are represented in Figure 2.

3.1 *Oxidative stress*

The main mechanism of NPs to produce oxidative stress is by the formation of reactive oxygen species (ROS). ROS molecules are formed from reactive intermediates which have strong positive redox potential. The different types of ROS formed by the NPs are: Singlet oxygen (O_2), superoxide radical (O_2-), the hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2). The ·OH and

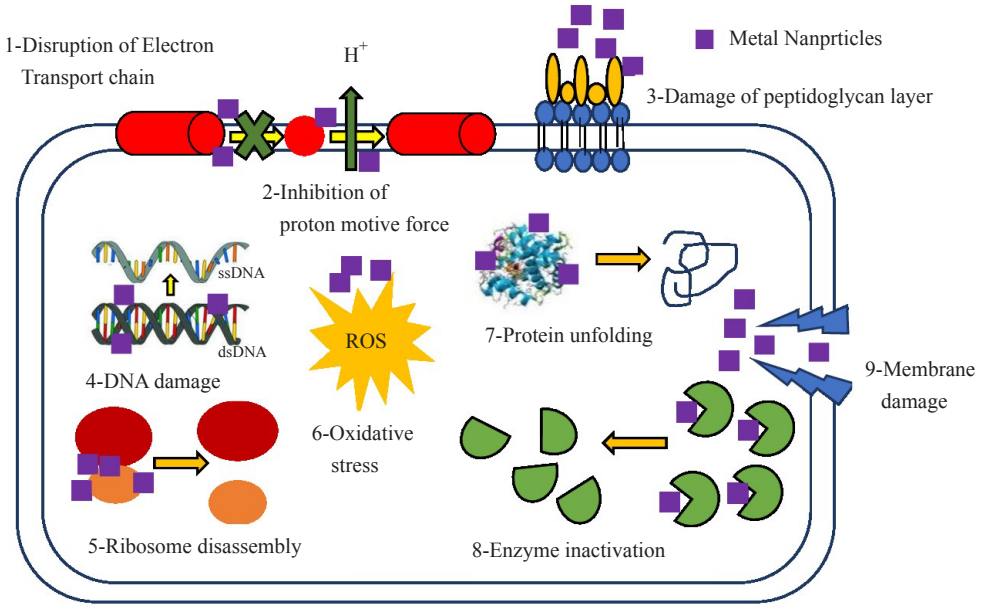

Figure 2. Schematic representation of the antibacterial mechanisms of Metal nanoparticles

O_2 leads to acute microbial death but O_2- and H_2O_2 cause less acute stress reactions, which can be overcome by superoxide enzyme and catalase antioxidants [43].

Oxidative stress is the main factor of membrane damage by affecting bacterial cell membrane permeability [44]. The Al_2O_3 NPs traverse the bacterial cell membrane, accumulate intracellularly and trigger loss of membrane integrity as a result of intracellular oxidative stress [45]. Nano silver ions prevent the proliferation of bacteria by activating the oxygen in water or air which leads to the formation of ROS and hydroxyl radicals [34-35]. Several studies have indicated that ROS play a main role in the interactions between DNA and bacterial cells [46]. ROS increase the expression of oxidative proteins, the main mechanism in apoptosis in bacterial cells [47]. Further, in bacterial cells, ROS can react with proteins and decrease the activities of some of the periplasmic enzymes, essential for the maintenance of physiological processes and normal morphology [48]. The production of ROS by NPs can occur by different mechanisms. TiO_2 NPs absorb light to generate electron-hole pairs which react with air and water on the surface of the NPs and produce ROS. Zinc NPs produce highly reactive ROS which are activated upon exposure to ultraviolet (UV) and visible light [49]. Superoxide and hydroxyl radicals of negatively charged ions present on the cell surface do not penetrate through the bacterial cell membrane, whereas H_2O_2 enters into the intracellular mileu by crossing the cell membrane. Quantitative real-time Reverse transcription polymerase chain reaction (qRT-PCR) showed that an increase in expression of *Kat* A and *Ahp* C genes were obtained which are related to ROS oxidative stress 52 and 7 times, respectively and the *Dna K* gene by 7 times. Under ultrasonic conditions, metal ions are released from cell surface which inhibit cell proliferation, as an effect of increased rates of transport of nutrients, oxygen, and waste products [50].

3.2 *Dissolved metal ions*

Metal oxide NPs slowly release metal ions, which are absorbed by the cell membrane and directly interact with amino (-NH), mercapto (-SH), and carboxyl (-COOH) groups of nucleic acids and proteins, and alter cell structure, inhibit enzyme activity, affect the normal metabolic functions, and eventually kill the microorganisms. However, the metal ions have lesser impact on the pH inside lipid vesicles demonstrating a decrease in antimicrobial activity. The main antimicrobial mechanism of NPs metal oxides is therefore not mediated by dissolved metal ions [51]. Superparamagnetic iron oxide NPs directly penetrate the bacterial cell membrane and interfere with the transfer of trans-membrane electrons. Heavy metal ions further act as carriers of antimicrobial agents by an indirect mechanism [52].

3.3 *Non-oxidative mechanisms*

Electron spin resonance (ESR), transmission electron microscopy (TEM), liquid chromatography-mass spectrometry (LC-MS), Fourier transform infrared (FTIR) analysis, Proteomic tools and flat cultivation have been employed to investigate the antimicrobial mechanism of MgO NPs. Three different types of MgO NPs have demonstrated efficient bactericidal activities on a strain of *E. coli* when exposed to natural light, UV and in total darkness. The antibacterial mechanisms of the three MgO NPs are not caused by membrane lipid peroxidation as a result of oxidative stress due to three main reasons: 1) when the surface pores are clearly visible by permeabilization of the bacterial cell membrane, MgO NPs are not observed, while, in energy-dispersive X-ray spectra, excessive Mg ions are not visible. Therefore, the antibacterial effect of MgO is presumably caused by cell membrane damage, 2) Only one type of MgO NP has been associated with production of ROS in small amounts, and, 3) treatment with MgO NPs does not significantly affect phosphatidylethanolamine (PE) and Lipopolysaccharide (LPS) in the cell wall, which suggests that lipid peroxidation is not the primary antibacterial mechanism. In addition, an increase in the amount of ROS-associated protein in the cell is not observed, while, there is a significant reduction in critical metabolic processes such as carbohydrate metabolism, energy metabolism, nucleotide metabolism and amino acid metabolism [18].

4. Factors affecting antibacterial mechanisms of NPs

The nanoscale parameters, which regulate antibacterial mechanisms are size, surface charge, zeta potential, morphology and crystal structure of NPs. Other factors such as environmental conditions, type of bacterial strain and exposure time also influence the antibacterial activity of NPs [53]. Metal oxide NPs with increased surface area and surface energy, and atomic ligand deficiency tend to exhibit aggregation. Therefore, the influence of important factors on the antibacterial action of metal oxide NPs is significant in order to elucidate the antibacterial mechanisms of NPs.

4.1 *Size*

The nanoscale features of NPs need to be considered for the assessment of antibacterial activity [54]. Formation of biofilms by bacterial adhesion makes the organisms highly resistant to conventional antibiotic therapy. The antibacterial activity of a NP is greatly affected by the size regime of the NP.

To prolong the release of drug against *S. aureus*, anodic oxidation process was used for adjusting the length and diameter of nanotubes. TiO_2 nanotubes and silica NPs show synergetic effects on their antibacterial activities. The mechanism and the extent of antibacterial activity are largely determined by the average size of TiO_2 nanotubes [55]. Smaller NPs have larger specific surface area and therefore have higher probability of crossing the bacterial cell membrane barrier than with larger NPs or polymers. The antibacterial effects of three types of $Mg(OH)_2$ NPs composed of nanoflakes correlated conversely with the sizes of the NPs attributed to the loss of integrity of cell walls. Further, the adherence properties of the three NPs of variable sizes on the bacterial surfaces increased the membrane permeability allowing antibacterial action [56].

4.2 *Shape*

The shape of NPs is one of the crucial factors to exhibit antimicrobial action. The differences in shapes of NPs show varying antibacterial effects through interactions with periplasmic enzymes [57]. When pyramid and sphere-shaped ZnO NPs were combined with β-galactosidase, the sphere-shaped ZnO NPs restructured the enzyme to produce photocatalytic activity, while the ZnO NPs in pyramid shapes showed prevention of the degradation of enzymes [58].

The antibacterial effects of Tb^{3+} activated Yttrium oxide nanophosphors (Y_2O_3:Tb^{3+}) have been studied for the first time by Prasannakumar et al. [59]. They have observed that prism-shaped Y_2O_3:Tb^{3+} NPs show specificity in antibacterial activity towards *S. aureus and Pseudomonas desmolyticum*. The direct contact between cell walls of the bacteria and prismatic Y_2O_3:Tb^{3+} NPs causes rupture of the bacterial cell membrane [59]. Therefore, shape is an important parameter in displaying antibacterial activity.

Ag NPs were firstly used as antibacterial agents in biomedicine due to their bactericidal activity. Cube-shaped Ag NPs exhibited more potent antibacterial activities than sphere-shaped and wire-shaped Ag NPs. This suggests that the shape of NPs can affect antibacterial activity due to their specific surface area and fast reactivity [60]. However, earlier studies have also shown that the shape of silver NPs does not influence microbial susceptibility [61-62].

4.3 *Roughness*

The effect of roughness of NPs on antibacterial action has been addressed by several researchers. The increase in roughness of nanoparticles facilitate the adsorption of bacterial proteins followed by reduced bacterial adhesion as demonstrated from Cu-nanoparticles functionalized thin-film composite polyamide RO membrane [63]. In another study, the roughness on the surface of TiO_2 nanoparticles enhanced antimicrobial activity using *Aspergillus flavus* as a reducing and capping agent [64].

4.4 *Zeta potential*

Zeta potential of NPs strongly influences bacterial adhesion. An electrostatic attraction force between the positively charged NPs and the negatively charged bacterial cell membrane results due to which positively charged $Mg(OH)_2$-$MgSO_4$ and $Mg(OH)_2$-$MgCl_2$ NPs are adsorbed on the bacterial surface [55]. The NPs have the ability to increase at the site of bacterial infection which helps to increase vascular permeability [65]. The accumulation of cationic NPs limits bacterial attachment and inhibits bacterial growth. The NPs penetrate the outer regions of *S. aureus* and act on bacterial cells to prevent their proliferation through ion exchange mechanisms [66].

The positively charged NPs enhance ROS production than negatively charged and neutral NPs. Negatively charged NPs do not adhere to bacterial cell membrane because both have negative potential. However, in higher concentrations, the negatively charged NPs effect antibacterial activity by molecular crowding resulting in interactions between bacterial surface and the NPs [67].

4.5 *Doping modification*

Clinically, the advantages of NPs are limited due to their aggregation behavior. Doping modifications of the NPs have been employed to prevent their aggregation and enable dispersion in aqueous media. Doping modification is an effective method for the regulation and control of the interactions between NPs and bacteria. ZnO/Au nanocomposites formed by doping of ZnO NPs with Au have been used for enhancing ROS generation and improve photocatalytic activity. The improved light absorption caused by the surface plasmon resonance wavelength of Au; the enhanced reactivity of photo-induced charge carriers due to an altered bandgap width of ZnO; efficient electron transport and charge carrier separation are some of the factors which increase the efficiency of NPs by doping modifications [68]. Doping of ZnO NPs with fluorine produces more ROS than their undoped form which results in strong antibacterial activity [69-70]. The oxide content on the surface of ZnO NPs is responsible for the regulation of broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria [71]. Nano-TiO_2 reduces the formation of biofilms due to its antibacterial activity and hence, is used widely in orthopedic and dental implants. In the case of doped TiO_2 NPs, their valence bandwidth is increased and forbidden bandwidth is reduced compared to undoped nano-

TiO_2, which improves its photocatalytic activity [72-73].

5. Bacterial factors affecting the effect of NPs

5.1 *Bacterial cell wall structure*

Cell wall gives shape, strength and rigidity and protects the cell from osmotic pressure and mechanical injury [74]. Based on the structure, components and functions of their cell wall, bacteria are classified as Gram positive (+) or Gram negative (-). The cell wall of Gram-positive cells contains a 20-50 nm thick peptidoglycan (PG) layer which is attached to teichoic acids [75]. Gram-negative cells contain structurally and chemically complex cell walls which comprises a thin PG layer and an outer membrane, covering the surface of the membrane. The outer membrane of Gram-negative bacteria renders resistance to hydrophobic compounds and contains lipopolysaccharides which confer structural integrity by increasing the negative charge of the cell membrane. The NPs exhibit pronounced antimicrobial activity against Gram positive bacteria than Gram negative bacteria. Gram positive bacterial cell wall forms covalent links with adjacent proteins and components forming a porous channel that allows the penetration of the NPs to effect antibacterial activity. On the other hand, the nonporous cell wall of Gram negative bacteria acts as a barrier and prevents the penetration of NPs to evade antibacterial action [76].

5.2 *Role of biofilm formation*

Surface-colonized microbes form biofilms which is a serious threat concerning economic and social issues and health perspectives [77]. Biofilms are complex secretions of extracellular polymeric substances (EPSs) (proteins, DNA, and exo-polysaccharide) produced by the microbial community which help cell wall adhesion to a solid surface (Figure 3) [78-79]. Bacteria can attach on the cell surface and move reversibly, but become irreversibly attached due to the expression of EPSs. Because of inhibition of the bacterial flagellum, the bacterial cells multiply rapidly forming a mature biofilm. The mature biofilms act as a barrier and protect the bacteria causing chronic infections [19]. Further, bacteria in the biofilms evade immune response by counteracting with their super-antigens. Hence, bacterial infections are a major cause of concern due to resistance to antibiotics and the host immune system. The electrostatic properties of NPs and the bacterial biofilms directly influence the interactions of the biofilms with the antimicrobial drugs. In most bacteria the biofilm matrices are negatively charged whereas in some bacteria like *Staphylococcus epidermidis*, the biofilms are polycationic [80].

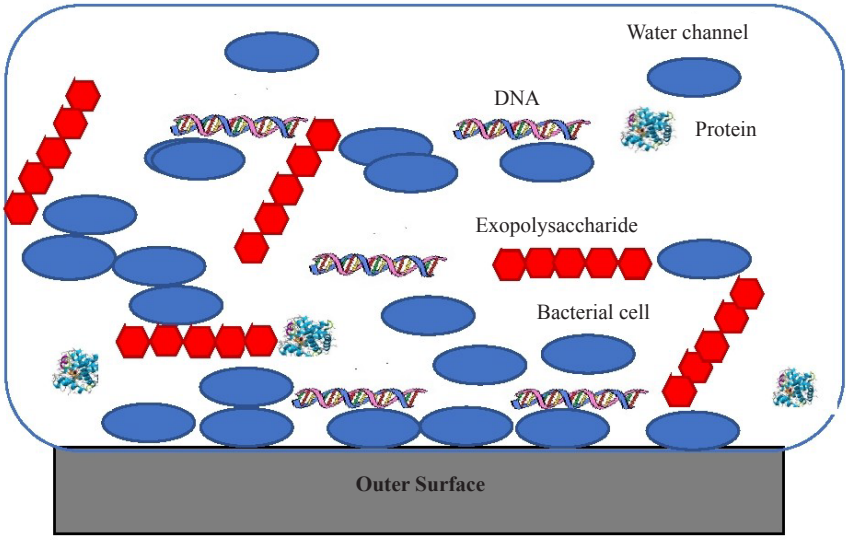

Figure 3. Structure of a mature biofilm

5.3 *Role of growth rate*

Bacterial growth rate is another factor affecting the tolerance against NPs. Slow-growing bacteria are more resistant to antibiotics and NPs than fast-growing bacteria [81-82]. The slow-growing bacteria exhibit tolerance property due to expression of the stress-response genes [83-84].

6. Applications of NPs with Antibacterial activity

Antimicrobial NPs are getting more focus from the research community because of the occurrence of drug-resistant bacterial infections worldwide and the excellent antimicrobial properties of NPs against MDR. NPs find several applications in different fields and each type of NP have unique properties that endow them with characteristic advantages. Many factors affect antibacterial activity and mechanism of NPs like shape, particle size, specific surface area, and surface curvature. The antibacterial applications of NPs are discussed in the following sections and is shown in Figure 4.

Over the past few decades, there has been a continual demand for Ag NPs for antimicrobial applications and its market size is expected to reach 6.6 billion by 2030 in the Asia-Pacific region, as published by Allied Markets Research (Globe NewsWire). Ag NPs have been extensively used as antimicrobials in several pharmaceutical and clinical sectors. Nano silver as colloidal silver nanoparticles as has been employed as a biocidal material registered in the USA since 1854. Since then, Ag NPs have been used in pharmaceutical applications to treat microbial infections commercially [85]. Ag NPs have been largely in demand in the medical sector and are chemically synthesized and marketed by several pharma industries globally. Samsung was one of the pioneering industries to launch Nanosilver for antimicrobial wash programs for household washing machines. Ag NPs are also gaining commercial exploitation in other industries.

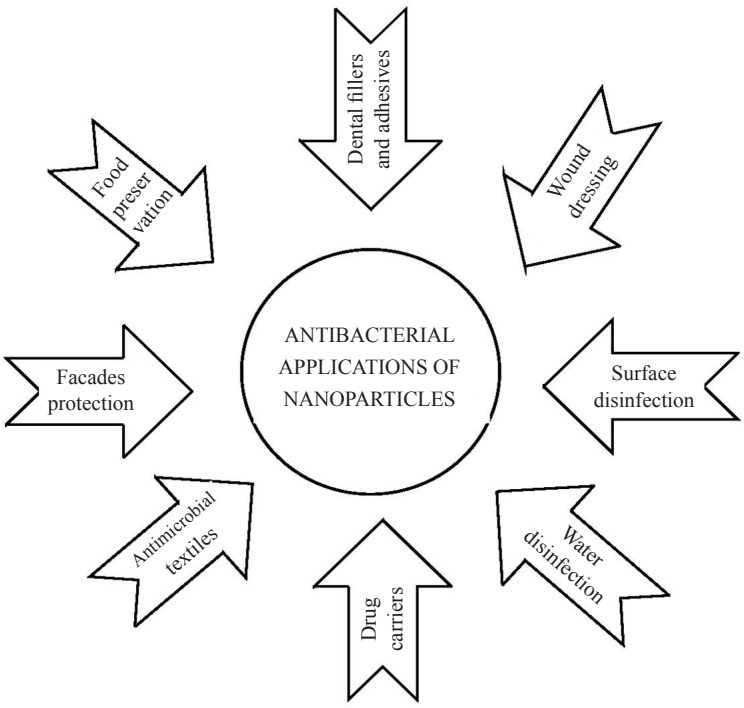

Figure 4. Some of the main applications of the antibacterial activities of Metal Nanoparticles

6.1 *Antibacterial coating of implants*

Biomaterials, despite their excellent mechanical strength and biocompatibility are vulnerable to many bacterial infections inside the body. Ag NPs have been directly coated on the metal implants by electrodeposition to treat microbial colonization which form biofilms [86]. The fully implantable devices, such as orthopedic, heart valves, dental implants and others are coated with antimicrobial coatings. The cardiovascular implants must have antimicrobial coating to be compatible to prevent thrombosis. The application of a titanium oxide coating on implants is based on pore morphology and its enrichment with silicon, phosphorus, calcium, and silver particles [87] which inhibit growth and bacterial adhesion and prevent inflammation around the implants. Nano coating also facilitates the adhesion and growth of osteoblast cell lines [88]. The intravenous and neurosurgical catheters which are partially implantable devices are increasingly susceptible to growth of bacterial colonies. Nano polymers can therefore be used to prevent bacterial infections by reducing biofilm formation in catheters. In case of invasive neurosurgical catheters, the risks of bacterial infection are significantly reduced by coating with NPs because of their sustained release over a period of 6 days killing the microbial organisms [89-90].

6.2 *Wound dressings*

Skin is the first protective line of defense and acts as a natural barrier by preventing the entry of pathogens and foreign substances. When the skin is damaged its natural functions are perturbed. Dressing is therefore required for application on the damaged skin to promote healing and prevent microbial infection. An ideal dressing material should have functions of the normal skin as well as enhance the wound healing process by formation of epithelial tissue, proliferation and migration of fibroblasts, reduction in scar tissue formation, and antibacterial and anti-inflammatory activities [91].

Wound infections are usually caused by Gram-positive bacteria and Gram-negative bacteria such as *Staphylococcus* sp. and *Klebsiella* sp. respectively. Generally, NPs act on Gram positive and Gram-negative bacteria as well showing broad-spectrum antibacterial properties which significantly inhibit overgrowth of bacterial species. A combination of nano silver with a mixture of polymeric compounds such as poly (vinyl alcohol) and chitosan (CS) has been used to treat wound infections. The increase in surface area of nano silver results in inhibition of bacterial growth and significantly increases the wound healing rate [92].

6.3 *Bone cement*

Bone cement is a self-curing plastic at room temperature which is composed of modified methyl methacrylate (MMA) or polymethyl methacrylate (PMMA) and is used in hip or knee replacement surgery to fix joint prostheses by filling the gap between bone and the newly replaced implant. In a previous study, as high as a 3% infection rate was observed following joint replacement surgery [93], while with the antibiotic-loaded PMMA, the infection rate decreased to 0% [94]. However, some studies have shown no reduction of infection with antibiotic loaded bone cement [95-96]. Therefore, the mixing of bone cement with antibiotics may not effectively reduce the infection rate after the arthroplasty surgery procedure. Currently, there is increased number of resistant bacterial strains, non-healing wounds and infectious diseases that are incurable which ultimately result in death. Antimicrobial NPs are currently a hot topic in research because of the efficiency of NPs that kill particular types of resistant bacteria [97]. Low concentrations of silver nanoparticles of about 0.05% have found to reduce bacterial infections after arthroplasty surgery, including MRSA (methicillin-resistant *S. aureus*), *S. epidermidis*, *S. aureus*, and *Acinetobacter baumannii* infections [98]. Silver nanoparticles are therefore being used to replace antibiotics during antibacterial bone cement preparations [99].

6.4 *Dental materials*

Dental plaque causes a favorable environment for the growth of bacteria in the mouth and are responsible for many common oral infectious diseases. Several dental materials with nano crystals are being used to treat many bacterial infections with improved efficiency. For example, a combination of Nano diamond-functionalized amoxicillin and gutta-percha is capable of eliminating residual bacteria after root canal filling for root canal treatments [100]. During

orthodontic treatment, an increase in bacterial proliferation and concomitant reduction in pH may cause dental plaques. Therefore, to inhibit bacterial infections by *S. mutans*, the dental brackets need to be coated with CuO and ZnO NPs [101].

Maxillofacial prostheses develop different bacterial infections by formation of biofilms and increase infections in the tissues causing inflammation in the maxillofacial areas. Therefore, nano-titanium dioxide need to be added to these prostheses and antibacterial actions occur upon exposure to light [102].

6.5 *NP-based antibiotic delivery systems*

Conventional antibiotic approaches in treatment of bone infection face a major problem of delivering the antibiotic at effective concentrations to the site of infection. In addition, high antibiotic doses lead to systemic toxicity. The use of NPs for bone infection is an efficient treatment methodology with bactericidal and osteogenic properties. NPs are therefore used as vehicles to deliver the antibiotics. Recent research is focused to deliver antibiotics by utilizing the biological efficiency of NPs [103-104]. The promising advantages of drug delivery systems are that they are biocompatible, biodegradable with controlled drug transport and efficiently deliver to the target site [105]. Gentamycin with CS/fucoid NPs act as a controlled release system to deliver antibiotics and antioxidants to treat pneumonia [106].

7. Clinical relevance of Antimicrobial NPs

Under laboratory conditions, it is often inappropriate to distinguish the action of a bacteriostatic or bactericidal agent against all bacterial microorganisms, while the classification offers potential information of the antibacterial agents in vitro. When considering pharmaceutical candidates, the compulsory requirement for treatment of infections should be its clinical outcome. In clinical settings, bacteriostatic agents for treatment of endocarditis, meningitis and osteomyelitis further require bactericidal activity. A good in vitro bactericidal aminoglycoside activity to treat *Salmonella enterica* serotype Typhi does not correlate with good efficacy in clinical observation [2]. Spellberg et al. [107] have called the medical community in the USA to devise measures to control the epidemic of antibiotic resistance infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) infection, drug resistance to *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* and multidrug resistance of Gram-negative Bacilli have been a serious concern for clinicians practicing in different fields of medicine. The infections causing morbidity and mortality in humans and multi-drug resistance strains pose a severe threat to US Public healthcare Agency, Infectious diseases Society and other Taskforce personnel. The increasing infections of MRSA, around 2.8 million drug resistance illnesses and about 35 million deaths in the USA annually has prompted researchers to work on Antimicrobial NPs research studies since the 20th century [108].

8. Effect of NPs on environment and ecosystems

Advances in the Nanomaterial sciences has led to enormous applications of NPs in biotechnology, medicine and allied fields and commercial products which result in accumulation of the NPs in soils and waterways affecting the surrounding environment. This causes harmful effects on microbes present in the ecosystem and disturbs the natural balance of the environment. The indiscriminate use of silver NPs releases Ag ions into the atmosphere harming the microbial communities in ecosystems [109]. Many microbes are critical towards maintenance of the environment and eventually the ecosystem, like bioremediation of soils and water bodies, element cycling, and nitrogen fixation to promote plant growth [110-112]. During nitrification, the symbiotic bacteria convert ammonium nitrogen to nitrite which is further oxidized to nitrate. Removal of these bacteria from the environment affect plant growth due to decreased nitrogen in soil [113]. As another example, when *E. coli* and MS2 phages are exposed to ZnO NPs and Ag NPs, increased transport of MS2 phages into *E. coli* from 2 to 6 times occur. Therefore, a number of phages can enter into bacteria and also there are more chances to transfer resistance genes to bacteria. This leads to serious public health risks by developing multidrug resistant bacteria [54]. To reduce environmental impact of NPs, they can be prepared using biological resources like plant seeds, leaves, stem etc., which also increases their potency. For example, Ag NPs have been synthesized using *Coccina grandis* leaf extract by a bio reduction mechanism which avoids the use of

hazardous and toxic solvents and have been found to exhibit photocatalysis [114]. In another study, an extract of *A. tamarii* MTCC 5152 was used for the synthesis of gold nanoparticles which reduced auric chloride. The myco-fabricated gold nanoparticles find applications in biological transformation and photocatalysis in the presence of NADH as a co-factor. *A. tamarii* MTCC 5152 could therefore be applied for production of gold nanoparticles as an environmentally viable and a cost-effective approach for application in dye biodegradation [115]. The phytocompounds from the leaves and twigs of *P.acidus* have been used for the synthesis of AgNPs as efficient capping and reducing agents. The AgNPs have shown to increase enzyme activity by 2- to 6-fold with excellent nanocatalytic activities on α-amylase, cellulase and xylanase than with pure enzymes [116].

Therefore, the scientific community should take precautions while using NPs and also find novel methods for environmentally friendly production and use of NPs. It is better to have a regulatory body to monitor the adverse effects and regulate the use of the NPs.

9. In vivo metabolism of NPs

In the case of Ag NPs in antimicrobial therapy, reports have demonstrated that Ag ions are deposited in skin epidermal layer, glomeruli of kidneys and intestinal tissue upon oral exposure [117]. Ag can react with reduced glutathione forming Ag-thiol polymer complexes, which undergo UV-photodecomposition to produce zerovalent Ag NPs. In visible light, these Ag NPs exhibit slower rates of antimicrobial activities [118]. Post oral dosing of Ag NPs after 8 weeks, Ag was found to be present in the brain and testes of rats [119]. In rat models, Ag NPs were found to be excreted in low amounts in urine and higher levels in faeces [120].

10. Toxicity effects of NPs

Human consumption of NPs as part of therapy has deleterious effects in the physiological system. Free silver ions can cause harmful effects in humans such as permanent bluish-grey discoloration of the skin (argyria) and eyes (argyrosis). Soluble Ag compounds cause liver and kidney damage, irritations in the eyes, skin and respiratory tract. Ag ions also cause observed changes in blood cells. Apart from these conditions, nano silver can cause oral toxicity, neurotoxicity and reproductive and immunotoxicity [121].

11. Conclusions

For many decades, antibiotics are misused and overprescribed due to which antibiotic resistance has become a major concern. Also, there is no new antibiotic groups discovered in recent years to treat infections. The lack of new antibiotics and an increase in resistant bacteria has become a public health risk. NPs are one of the emerging antimicrobials due to their efficiency to treat resistant microorganisms. The NPs work in different mechanisms compared to traditional antibiotics which are currently used. Due to their multi-target mechanisms, NPs can be used to treat MDR bacteria in near future. Before treating NPs in clinical practice, more research has to be done like standardization of formulation, characterization and testing. In addition, some studies examine the effect of NPs in human cells, but more data is needed for cytotoxicity and immune responses of the NPs. The NPs should not be cytotoxic to humans but it should have desirable antimicrobial activity. In practical uses, higher dosages of the effective concentrations of antibacterial agents than their cytotoxic levels can lead to serious contraindications. In conclusion, NPs can become future antimicrobials after standardized practices in NP fabrication considering maximal validation. There is also the need for regulatory body for the use of NPs in various sectors. The long term pros and cons has to be calculated, like their toxicity, side effects, immune reactions on system and their impact on environment and different ecologies. Thereby, more importance has to be given for studies on prevention of resistance in bacteria using NPs.

Aknowledgements

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Conflict of interest

The authors declare no competing financial interest.

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