Chapter 6
Application of Metal and Metal Oxide Nanoparticles as Potential Antibacterial Agents

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Abstract With the widespread use of antibiotics, the bacteria have evolved to build up mechanisms to resist the activity of antibiotics. The antibiotics target cell wall, protein synthesis and DNA replication, whereas microbes resist it through genetic basis or mechanistic basis of environmental resistance. The nanoparticles have shown a great potential as an alternative to antibiotics for treatment of microbial infections. Nanoparticles cause microbial inactivation via oxidative stress, dissolved metal ions or non-oxidative mechanisms and target cell barrier, bacterial protein, enzymes and DNA synthesis and its metabolism. The multiple mechanisms which nanoparticle employ simultaneously against microbes can have the potential to overcome the microbial resistance by providing the bacteria insufficient time to mutate and develop resistance. This book chapter focuses on mechanisms of antibiotic action and resistance developed by bacteria. Various metal nanoparticles (silver, copper, gold, aluminium) and metal oxides nanoparticles (copper oxide, titanium dioxide nanoparticles, zinc oxide and magnesium oxide nanoparticles) that are used for antibacterial action and impact of physicochemical properties are discussed. The chapter also elucidates the potential mode of action by various nanoparticles and impact of various important factors such as size, composition, shape, morphology, zeta potential and environmental conditions on antibacterial effectiveness.

Keywords Antibiotic action · Antibiotic resistance · Nanoparticle · Antibacterial action · Oxidative stress

6.1 Introduction

The field of nanotechnology has emerged as one of the most extensively researched fields involving multidisciplinary branches of science. It has been one of the major growing sectors in scientific field of research and also has large scale implication

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in industry, hence often called as next generation revolution. The implications of nanotechnology extend to the environmental field, which has benefits that can be categorized into treatment and environmental remediation, pollution sensing and detection and prevention of pollution. Nanoparticles (NPs) can have applications in soil, wastewater treatment, groundwater treatment and also large part in air remediation (Yadav et al. 2017).

Ever since the discovery of antibiotics as antimicrobial agents, they have been used extensively to treat bacterial and fungal diseases. Use of antibiotics comes with two advantages: first it has been cost-effective and another it has given powerful outcomes (Wang et al. 2017). But with the increasing use of antibiotics, the bacteria are artificially selected and acquiring resistance against these substances. In fact, there has been evidences of bacteria, which have developed resistance to multiple drugs via various mechanisms of gene transfer (Hajipour et al. 2012).

With the emergence of these drug-resistant bacteria, it can prove fatal in future, since the resistant properties are evolutionary based or can be developed spontaneously (Wang et al. 2017). In many cases, the last generation of drugs is being used for treatment. If anyhow, there is a development of resistance in those bacteria, we would be left with no option and epidemic may break out. Even with the immense progress in the field of medicine, the rate of mortality and morbidity that has been caused by bacterial diseases is large in numbers (Rai and Bai 2011). Therefore, there is an urgent need for an alternative for treatment of microbes. So, that the last generation of antibiotics can be preserved for future urgency.

If we go through the ancient literature, we can find that they have been using several inorganic elements such as copper, silver, gold and other materials to combat the effects of microbes. The idea has given origin to the use of inorganic nanoparticles as a potential antimicrobial agent, which can extend in various fields such as agriculture, food industry, soil and wastewater remediation, textile industries and other numerous applications (Rai and Bai 2011).

Nanoparticles are small particles, which have a range of scale 1–100 nm and have at least one of its unit in this scale. The inorganic and organic nanoparticles have been found as having potential antimicrobial properties. Metallic nanoparticles of inorganic origin have been profoundly used and experimented with the microbes and are successful in either killing or stopping its metabolism or reproduction hence restricting the growth. These nanoparticles have shown to have effect on a broad spectrum of both gram-positive as well as gram-negative bacteria (Wang et al. 2017). This success of inorganic nanoparticles can be attributed to its property to endure the adverse conditions and the unique property of nanoparticles, which it possesses due to its small size and large surface area. These factors provide advantage to kill bacteria, as nanoparticles can show surface phenomenon of killing instead of going inside the cell to disrupt it. This property can also be hypothesized as it can give the bacteria a very little time to develop resistance against either antibiotic coated with nanoparticles or nanoparticles alone (Rai and Bai 2011). Hence, we can think of a probable solution to the antibiotic resistance complication. Nanoparticles can also be made as composites such as to tackle the bacterial resistance with more than one mechanism hence rendering it viable for destruction (Wang et al. 2017). This book

chapter discusses in details various aspects of antibiotics action and mechanism of antibiotic resistance development. Additionally, also the chapter discusses various types of nanoparticles that can be used for antimicrobial activities and their mode of inaction potential for various biological agents.

6.2 Mechanism of Antibiotic Action

Antibiotics act as powerful medicines to fights infectious diseases. Their action is such that either they stop the bacteria from reproducing or they kill or destroy the bacteria. In humans, the immune system consists of white blood cells (WBCs) that is responsible for attacking infectious bacteria and fighting the infectious diseases but sometimes these harmful bacteria become too large in number, in this case the immune system fails to cope up with this number, antibiotics comes in to aid in this condition (Munita and Arius 2016).

There are two types of bacterial found in environmental systems: gram positive and gram negative (Fig. 6.1). The figure illustrates bacterial cells are of two types,

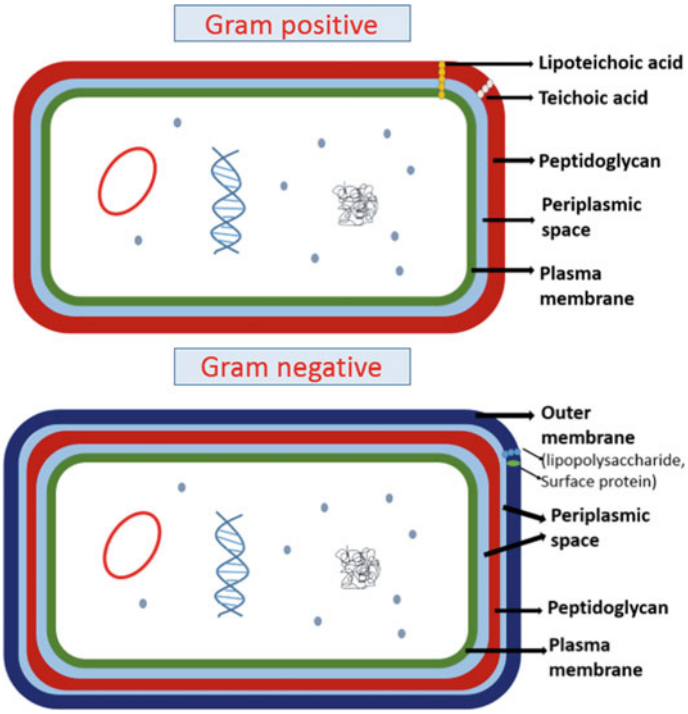

Fig. 6.1 Structure of bacterial cell envelope. Adopted from Kapoor et al. (2017)

gram positive and gram negative. Gram-positive bacteria consist of thick rigid peptidoglycan cell wall around the cell membrane that is attached to teichoic acids found only in gram-positive bacteria (Scott and Barnett 2006), whereas gram-negative bacteria have a thin peptidoglycan cell wall which is surrounded by second lipid membrane, the outer membrane (OM). The space between the outer membrane and the cell membrane is called periplasmic space consists of periplasm. Outer membrane provides additional protective layer against foreign substances and prevent them to enter to into bacterium (Munita and Arius 2016; Kapoor et al. 2017).

Outer membrane however also contains porins that allow entry of various molecules such as drug. Cell wall is a tough layer which provides the bacterium its characteristic shape and also prevent it from mechanical and osmotic stresses. The function of cell membrane is to prevent ions from flowing out or into the cell and also maintains cytoplasmic or cellular bacterial components in a defined space (Munita and Arius 2016).

The mechanism of an antibiotic action takes place in a biochemical way such as the drug works effectively. Antibiotics can be target-specific like a drug that binds like an enzyme (e.g. antibiotic or a receptor). Mechanism of action of antibiotics can be described as the biochemical process especially at a molecular level. (Munita and Arius 2016; Mazel and Davies 1999).

Classification of antibacterial agent is done on the basis of their activity spectrum, impact on bacteria and their way of action. Activity spectrum of antibiotics can be further divided into broad spectrum antibiotics which are effective against both the gram-negative and the gram-positive bacteria and narrow spectrum antibiotics which have restricted activity, their primary activities are against microorganism of only a particular species (Munita and Arius 2016; Kapoor et al. 2017), e.g. glycopeptides and bacitracin are effective against gram-positive bacteria, polymixin that is effective against gram-negative bacteria, aminoglycosides and sulphonamides are effective against aerobic organisms and nitroimadazoles against anaerobes (Kapoor et al. 2017). However, this spectrum of antibacterial activity may change if the bacterium acquires resistance genes.

Effect of antibiotics on bacteria involves different mechanism of individual antibiotics on respective bacteria therefore antibacterial has different effects on bacterial agents and it is either bacteriostatic or bactericidal. The latter consists of drugs that can kill bacteria (e.g. cephalosporins, aminoglycosides, quinolones, penicillins) whereas the former consists of drugs that inhibit or delay the bacterial activity of growth and their replication (e.g. tetracyclins, macrolides, sulphonamides). Some antibiotics can act as both bactericidal and bacteriostatic which depend upon the dose given, the duration of exposure and the original state of invading bacteria, e.g. fluoroquinolones, aminoglycosides and metronidazole, and they can kill bacteria depending upon its concentration, higher the concentration higher is the rate of killing. (Hajipur et al. 2012; Kapoor et al. 2017). Antibiotics can also show antagonistic and synergistic effect on each other like action of aminoglycosides is enhanced with antibiotics that inhibit cell wall synthesis (Hancock 2005).

Antibacterial action generally involves three mechanisms which involve inhibition and regulation of enzymes involved in the pathway of cell wall biosynthesis,

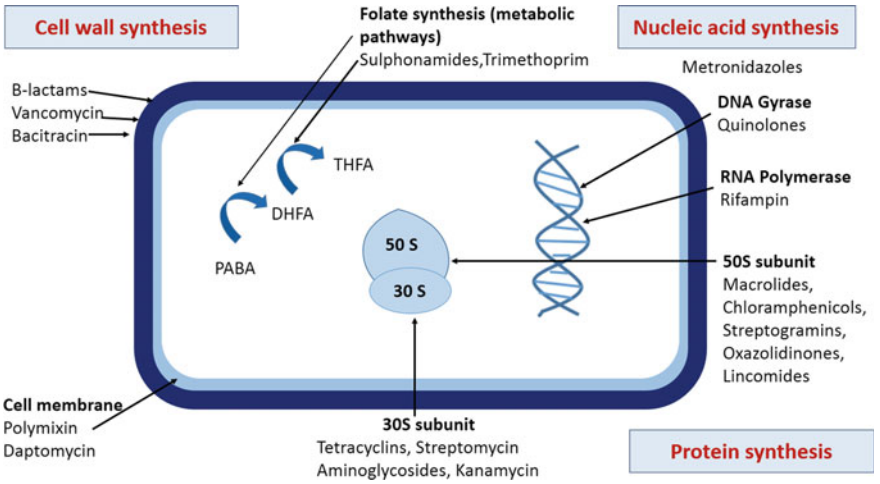

Fig. 6.2 Mechanism of antibiotics action. Adopted from Kapoor et al. (2017). *Note* PABA: Para-aminobenzoic acid, DHFA: dihydrofolic acid, THFA: tetrahydrofolic acid

metabolism of nucleic acid as well as deoxyribonucleic acid (DNA) repair or pathway of protein synthesis and disturbance in bacterial cell membrane structure. The detailed mechanisms are shown in Fig. 6.2. (Kapoor et al. 2017). Multiplying cells are the main target for antibiotics as these cells have the most of these cellular function. Sometimes there is overlapping in these functions that occur between prokaryotic bacteria cells and eukaryotic mammalian cells. Some antibiotics have also been function as anticancer agents (Munita and Arius 2016).

6.2.1 Antibiotic Resistance in Bacteria

Antimicrobial resistance has developed over a period with interaction with many organisms and their environment. Mostly antimicrobial compounds were naturally produced molecules and as such the bacteria that were co-resident evolved mechanisms to resist and overcome the action of antimicrobials to survive this gave rise to the organisms that often considered as "intrinsically resistant" to more than one antimicrobials (Munita and Arius 2016). In general, we refer to the expression of the "acquired resistance" in particular bacteria, which was previously susceptible to antimicrobial compound. The antimicrobial resistant microorganisms can be either attributed to either mutation in their genetic material or horizontal transfer of the gene from some intrinsically resistant microorganism present in the environment (Mazel and Davies 1999; Munita and Arius 2016). Another important factor in antimicrobial resistance or susceptibility is the effectiveness of antimicrobial agents on microbes

which is a relative phenomenon involving multilayers of complexity (Munita and Arius 2016).

Bacteria can adapt to a wide range of environmental conditions; this genetic plasticity allows them to develop mechanism to resist the action of antimicrobial that can jeopardize their survival. Bacteria share similar ecological niche with antimicrobial producing organisms. Hence, in time bacteria evolved to withstand the negative impacts of antibiotics and subsequently this intrinsic resistance helped to survive in its presence. If we consider an evolutionary perspective, then bacteria developed two major mechanisms in genetic strategies to combat this effect. First is mutation at gene(s) level which is generally associated with action of the compound on microbes. Second is horizontal gene transfer (HGT), i.e. acquisition of foreign DNA that codes for resistance coding genes (Kapoor et al. 2017; Hopwood 2007).

Generally, these mutations effect the antibiotic resistance by altering antibiotic action by one of the following mechanisms as described in Fig. 6.3, modifying the antimicrobial target, i.e. decreasing affinity for the drug, decreasing uptake of drug, extrusion of harmful molecules by activating efflux mechanisms, modulation of regulatory networks and changing important global metabolic pathway. Therefore, resistance arising due to acquired mutational changes in diverse groups and varies in complexity (Singh et al. 2014; Wright 2011).

As the bacteria share the same ecological niche with the antimicrobial producing agents, they develop resistance in themselves, and this "environmental resistome" gives a robust source for other bacteria for acquisition of resistance genes. HGT can occur through three ways, transformation, which occurs through incorporation

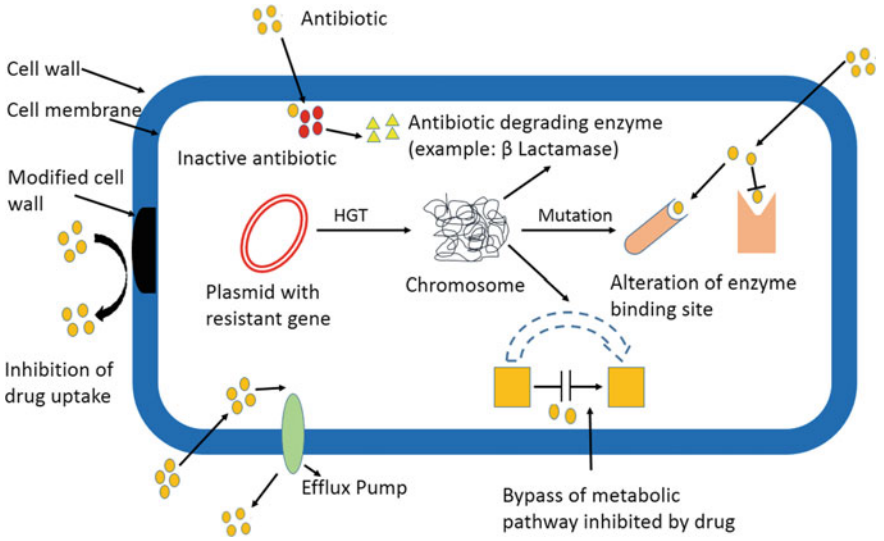

Fig. 6.3 Mechanisms of antibiotic resistance. Adopted from Singh et al. (2014)

of naked DNA, transduction which is phage mediated and conjugation which is considered as bacterial sex.

Among the three transformations is simplest way of acquisition of genes, but only few species of pathogenic bacteria are naturally able to incorporate naked DNA and develop resistance (Munita and Arius 2016). Bacteria have evolved over millions of years of evolution and have developed sophisticated mechanisms towards antimicrobial molecules. The resistant microbial class usually has attained this characteristic property via multiple biochemical pathways whereas a bacterial cell can achieve this resistant property by using more than one mechanism. The biochemical route for the attaining antibiotic resistance can be categorized as, modifying the antimicrobial molecule, preventing the antibiotic from reaching its target (either by decreasing its penetration or by active efflux of antimicrobial compound), changing and/or bypassing of the target sites and resistance through global cell adaptive processes. Every category mentioned can have multiple specific biochemical pathways (Kapoor et al. 2017; Munita and Arius 2016).

6.3 Nanoparticles Antibacterial Application

The detail mechanistic path of NPs interaction with bacteria and various types of NPs used for antibacterial are discussed along with different physicochemical properties that are critical for effective antibacterial action.

6.3.1 Interaction Mechanism of Nanoparticles with Microbes

There are different types of nanoparticles such as metallic, metal oxides, doped oxides, composites with different size, morphology and composition. Depending on the nanomaterial's properties like size and composition different NPs like copper, gold, silver, aluminium, zinc, magnesium and titanium, nanoparticles will show different effectiveness. These nanoparticles have different mechanism of action as bactericidal, antiviral and antifungal agents. Cell wall and cell membranes act as essential defensive barriers and protect the bacterial cell from osmotic and physical pressures, therefore plays a crucial role in the maintenance of the shape of bacterium. The gram-positive and gram-negative bacteria differ in their cell membrane's components hence have different adsorption pathways for NPs. Gram-negative bacteria have a unique structure lipopolysaccharide (LPS) which provides a negative charge, and it is responsible for attraction of NPs, whereas in gram-positive bacterial cell wall consists of teichoic acid, so NPs get dispersed along the molecular chain of phosphate, and this prevents their aggregation (Hajipour et al. 2012; Wang et al. 2017). The mechanism of NPs bactericidal action also depends on structure and components of bacterial cell. The mechanism of NPs penetration was through diffusion and adsorption (Melander et al. 2018; Slavin et al. 2017).

There are several mechanisms that were proposed as the emergence of NPs as antimicrobial agent. For example, bacterial cell metabolism can be changed by using metal NPs. The NPs have a promising potential as a cure for bacterial diseases. The NPs have shown their ability to enter biofilms and inhibit its formation shown by Ag-inhibited expression of genes (Wang et al. 2017). NPs to carry out their antibacterial action must come in contact with the bacteria. This contact can be in the form of electrostatic attraction, interaction between receptor–ligand, van der Waal force and hydrophobic interactions (Hajipour et al. 2012). NPs after crossing bacterial membrane can affect the metabolism of bacterial cell, influencing a change in its shape and the cell membrane's function. The natural bacterial metabolism cycle plays an important role in sustaining bacterial growth and development and in addition can also be a cause of disease. Bacterial metabolism disruption damages the membrane of bacterial cell and induces oxidative stress, eventually leading to death of bacterial cells (Wang et al. 2017). Further, NPs can also react with bacteria's genetic components like DNA, ribosomes, lysosomes and interfere with enzymes which can lead to oxidative stress, change in permeability of the cell membrane, heterogeneous alteration, disorder in electrolyte balance, inhibition of an enzyme, deactivation of a protein and interfere with the expression of genes as shown in Fig. 6.4 (Singh et al. 2014). CuO NPs influence was studied on bacterial denitrification. It was seen that there was significant alteration in the expression of the key proteins. CuO NPs lead to interference with the proteins that were involved in the nitrogen metabolism, transfer of electrons and transfer of substance (Pelgrift and Friedman 2013). But the following three mechanisms have been mostly proposed and followed in research. They are oxidative stress, release of metal ions and non-oxidative mechanisms.

Among NPs antimicrobial mechanisms, one of the important ways is production of reactive oxygen species (ROS)-induced oxidative stress. ROS are those molecules

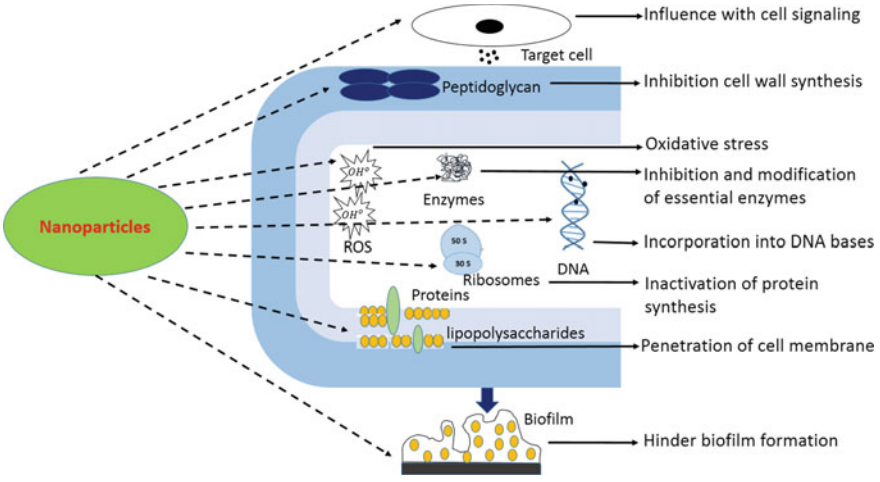

Fig. 6.4 Mechanisms for antimicrobial activity of nanoparticles. Adopted from: Singh et al. (2014)

with reactive intermediate having a strong positive redox potential, and various other types of ROS were produced by reduction of oxygen molecules using different types of NPs. There are generally four types of ROS exhibiting different dynamics level and activity, they are superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^o) and singlet oxygen (O_2) (Dwyer et al. 2009). Studies have showed that (OH^o) and O_2 have a more potent bactericidal activity as compared to O^{2-} and H_2O_2 as they may be neutralized by various endogenous antioxidants like catalase and superoxide enzymes (Wang et al. 2017). TiO_2 nanoparticles let the bacterial DNA's compression, degeneration and fragmentation, thus reducing physiological activity of the genes. The affinity and mode of binding of TiO_2 NPs with DNA were predicted using molecular docking, that showed TiO_2 NPs targets G:C-rich DNA. Further, analysis of the whole genome was used to identify the molecular mechanism of the bacterial apoptosis (Wang et al. 2017). In normal conditions, the ROS production and its clearance inside the bacterial cell are in balanced condition, but with the excessive ROS production oxidation is favoured by the redox balance in cell. The oxidative stress is thus produced by this unbalanced redox balance, that leads to the damage of individual components of the cell (Nel et al. 2006).

Metal oxides release their ions in the environment slowly that are absorbed by the bacterial cell membranes, and these metal ions can directly interact with the proteins and nucleic acid's functional groups like mercapto (–SH), carboxyl (–COOH) and amino (–NH) groups, causing damage to enzyme activity, disruption of cell structure, interfering with normal physiological metabolic processes and finally inhibiting the microorganisms (Wang et al. 2017). Metabolic pathway of bacteria is not isolated, but is incorporated into living cell's complex activity. Therefore, deliberate alteration of the bacterial metabolism can be utilized to regulate the pathogenicity of bacterial cell. Various mechanisms for the effects of nanoparticles on bacterial metabolism have been suggested, including a mechanism for reactive oxygen and a mechanism for metal ion dissolution (Melander et al. 2018).

6.3.2 Different Types of NPs and Their Action

There are different types of metallic nanoparticles like copper, gold, silver, aluminium, zinc, magnesium and titanium nanoparticles. These nanoparticles have different mechanism of action as bactericidal, antiviral and antifungal agents. Some of the metal and metal oxide nanoparticles and their activity are briefly discussed as follows:

Silver Nanoparticles

Silver nanoparticles have a wide range of applications in medical field like in treatment of wounds, burns and infections. The salts derived from silver and in its various forms are found to be antimicrobial in nature. It is also reported that Ag nanoparticles can be used as medium for delivering the antibiotic to target sites, and there is a possibility of antibiotic coating with Ag to enhance antibiotic activity (Wang

et al. 2017). Multiple studies have been conducted to explain the mechanism of Ag nanoparticles as microbial growth inhibitors. According to one of the mechanisms, Ag nanoparticles show high affinity for sulphur moiety and phosphorous moiety of proteins and genetic elements in bacterial cell, Ag nanoparticles react with these moieties of protein and effects viability of cell of the bacterial or it can also interfere with enzyme action. Ag nanoparticles also react with the phosphorous of genetic material (DNA mostly) and inactivate its replication, hence stopping its growth and reproduction (Pelgrift and Friedman 2013; Qu et al. 2013). Silver NPs also have the ability of targeting bacterial membrane ultimately that causes dissipation of proton motive force that leads to blockage of oxidative phosphorylation (Singh et al. 2014). It was reported that Ag nanoparticles with a characteristic size which has less than 20 nm diameter are able to attach itself to the sulphur moiety of proteins on cell membranes which ultimately makes it more permeable and finally bacterial death (Rai and Bai 2011). Silver NPs have ability to modulate bacterial signal transduction. Ag^+ ions have the ability to interact with thiol groups of important enzymes causing their inactivity leading to the disruption of cellular functions, and this can collapse the membrane potential and inhibit ribosomal binding to the ribosomal ribonucleic acid (rRNA) (Singh et al. 2014). NPs that are within the size of 10–15 nm have been found to show concentration dependent effects against both the groups of bacteria (gram positive and negative). At microlevel concentration, it has showed effect such as in oxidative phosphorylation, uncoupling of respiratory electron transport chain, interfering with the permeability of bacterial cell membrane with respect to the exchange of phosphate and protons or inhibition of enzymes of respiratory chain. Further increase in concentration of Ag^+ ions has shown effects on cytoplasmic components and genetic materials (Rai et al. 2009). Ag NPs smaller than 10 nm were found to be toxic for *E.coli* and *Pseudomonas aeruginosa* (Li et al. 2008).

A study of silver NPs effect on bacterial cell morphology was done which on *E.coli*. The results of Ag^+ ions showed similar effects on morphology of bacteria. It detached the cell wall from the cell membrane (Choi et al. 2008). It was found out that the gram-negative bacteria had more inhibitory effect as compared to the gram-positive bacteria. This might be due to the composition of gram-positive bacteria that has a thick peptidoglycan layer in cell wall which might prevent it from the inhibitory action of silver ions (Rai and Bai 2011). The silver and sulphur ions form dense granules of electrons inside the cytoplasm which suggest that Ag NPs may have interacted with the nucleic acids and somehow impaired the DNA replication that lead to the cell viability loss and finally death of cell (Rai et al. 2009). Ag NPs have also proved to inhibit biofilms (Sheng and Liu 2011).

Ag NPs of size 2–5 nm using integrated with green fluorescent protein (GFP) in E. coli was studied. It was found that Ag NPs below 100 nm caused perforation in cell wall via getting attached to it and lead to bacterial death. Studies showed the activity of Ag NPs on bacteria were size- and shape-dependent. An experiment on E. coli was done using truncated triangular-, spherical- and rod-shaped Ag NPs, and the maximum effect was seen in case of triangular nanoplates. The smaller the particle higher was its antimicrobial activity (Wang et al. 2017). Silver NPs produced from biosynthetically from fungus, plants extract and bacteria have shown strong

antimicrobial efficacy against multidrug-resistant (MDR) mycobacterium tuberculosis (Singh et al. 2014). Agarwal et al. (2013) studied the effect of biosynthesized silver nanoparticle against standard *Mycobacterium tuberculosis* along with 26 other chemical isolates that induced "multidrug resistance (MDR), drug sensitive (DS), extensive drug resistant (XDR) and mycobacteria other than tuberculosis (MOTT) strains" and found out they have effective bactericidal activity. Ag-ACF/CNF (silver nanoparticle composite with activated carbon nanofibres (ACF) or carbon nanofibres (CNF)) was found to have lethal inhibitory effect on bacteria *E.coli* and *S.aureus* that were completely inhibited in duration of 72 h (Singh et al. 2013; Singh et al. 2014). Silver nanoparticles synthesized in the presence of sesame oil cake were found to have good inhibitory effect on gram-negative bacteria like *P. aeruginosa, K. pneumoniae* and *E.coli* (Alfuraydi et al. 2019).

Copper Oxide and Copper Nanoparticles

CuO has a monoclinic structure and semiconducting in nature. Among the copper compound family, it is considered as the simplest with potentially advantageous properties with respect to superconductivity, high temperature effects on electron correlation and with spin dynamics which makes it valuable for large array of applications (Rai and Bai 2011). The crystal structure of CuO possesses photovoltaic and photocatalytic properties as well as photoconductive functionalities (Santo et al. 2008). CuO is used as antimicrobial agent as it is cheaper compared to that of silver, readily mixes with the polymers and comparably stable if we consider its physical and chemical properties which make it useful in wide applications. CuO as ionic nanoparticle can act as potent antimicrobial owing to its unique crystal morphology and high surface area such as its active action against a range of hospital acquired infection but is concentration dependent (Nel et al. 2006). It has been hypothesized that if the amount of peptidoglycans (negatively charged) is reduced, gram-negative bacteria (*Pseudomonas aeruginosa* and *Proteus spp.*) become relatively less vulnerable to those positively charged NPs. However, combined NPs of CuO and Ag showed greater activity towards gram-negative strains in time-kill experiments (Haipour et al. 2012). Studies suggested that for the optimal activity as antimicrobials, local environments need the presence of ions in it. *B.subtilis* have been found to susceptible towards Cu NPs action as the affinity of Cu is higher towards the amines and carboxyl groups that are abundant on its cell surface (Huh and Kwon 2011). Copper ions can intercalate with the nucleic acid strands and interact with DNA molecules. It can also disrupt the biochemical processes by getting inside the cells of bacteria (Melander et al. 2018).

The mechanism of action of Cu and Cu-based nanoparticles is reported to be associated primarily to the large surface area-to-volume ratio of the nanoparticles (Pramanik et al. 2012). Cu nanoparticle-specific mechanism of action on bacterial cells is mainly threefold, as shown in Fig. 6.5. Firstly accumulation and diffusion of the nanoparticles through the bacterial cell membrane, subsequently altering the permeability of the cell membrane by blocking or inducing excess release of one or several lipopolysaccharides, membrane proteins and transmembrane proteins which may constitute one mechanism of cell rupture. Secondly, the nanoparticles may

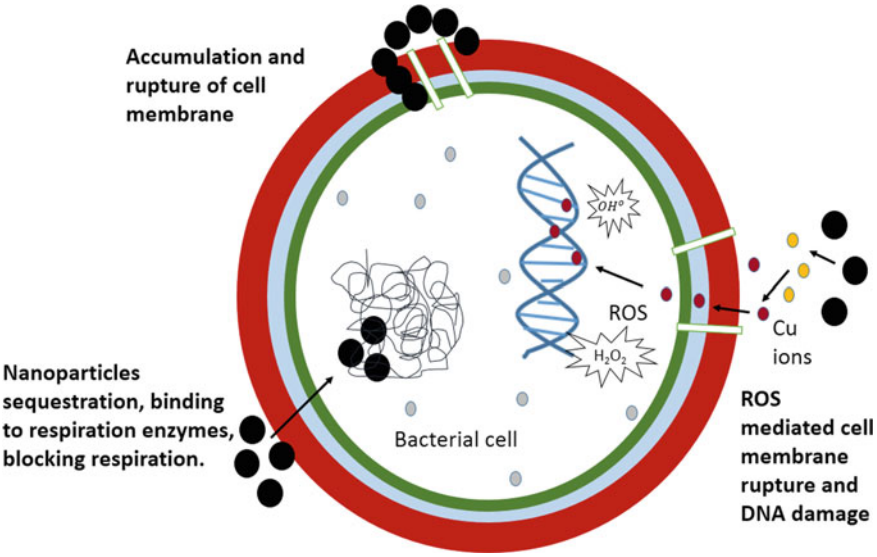

Fig. 6.5 Known mechanisms of action of Cu and CuO nanoparticles on bacteria. Adopted from Chatterjee et al. (2014)

induce release of their constituent metal ions, which may trigger a host of reactive oxygen species (ROS) generation, leading to oxidative damage of the cell membrane and DNA damage. The third reported mechanism of action of Cu-based nanoparticles involves the sequestering of the nanoparticles inside the cell, following which the nanoparticles bind to specific enzymes, blocking respiration (Chatterjee et al. 2014).

The nature and antimicrobial efficacy in a designed nanoparticle system may include one or a combination of these mechanisms. Additionally, the mechanism of action is also dependent on the size, shape, charge, coating and crystallinity of the nanoparticles apart from the nature of the microbes (Chatterjee et al. 2014; Wang et al. 2017). Some studies also indicated the broad spectrum activity of Cu-based nanoparticles, by demonstrating their mode of action by interaction and subsequent distortion and damage of phosphorus and sulphur containing biomolecules, such as DNA and proteins, respectively (Ruparelia et al. 2008). The hydroxyl radicals produced by copper ions and metals were found to damage the DNA and necessary proteins of *E.coli* (Baek and An 2011). Cu-ACF/CNF was found to have lethal inhibitory effect on bacteria *E.coli* and *S.aureus* (Singh et al. 2013; Singh et al. 2014).

Titanium Dioxide Nanoparticles

The most widely used photocatalyst is TiO_2 because of its low cost, high photostability and efficiency in generating reactive oxygen species (Singh et al. 2014). The semiconductor TiO_2 is utilized as a photocatalyst to induce a series of reductive and

oxidative reactions on its surface (Musee et al. 2011). TiO_2 is shown to have antimicrobial activity hence it is utilized in various applications, such as water disinfection, paints, protection of marbles from microbial corrosion, coating on wood, fabrics, food packing films and as a surface disinfectant (Foster et al. 2011). There are mainly three crystal phases of TiO_2 referred as anatase, rutile and brookite. Composition of these crystal structures is shown to have an impact on its photocatalytic properties (Mahmoud et al. 2018; Mcwan et al. 2011).

The bandgap of TiO_2 is reported as 3.2 eV. TiO_2 shows photocatalytic activity when it is illuminated with ultraviolet light at wavelength less than 385 nm, this gives it strong oxidizing power and is the basis of its inhibitory activity and bactericidal action. When photoexcited by UV light TiO_2 particle generates Reactive oxygen species (ROS) which are responsible for the killing of bacteria as shown in Fig. 6.6 (Sahu et al. 2011). The effect of photocatalysis has been studied on *E.coli* present in water. TiO_2 NPs show photocatalysis which can provide an alternative means to self-disinfect surfaces that are contaminated and its further application can be extended to find out potential disinfecting solutions to prevent formation of biofilm, which can be used in food processing industries (King et al. 2018). TiO_2 has been found to have effective bactericidal action on *E.coli* which has been the basis of development of the photocatalytic methods for bactericidal action against bacteria in aqueous environment (Sunanda et al. 1998). There has been studies on the effect of TiO_2 NPs which

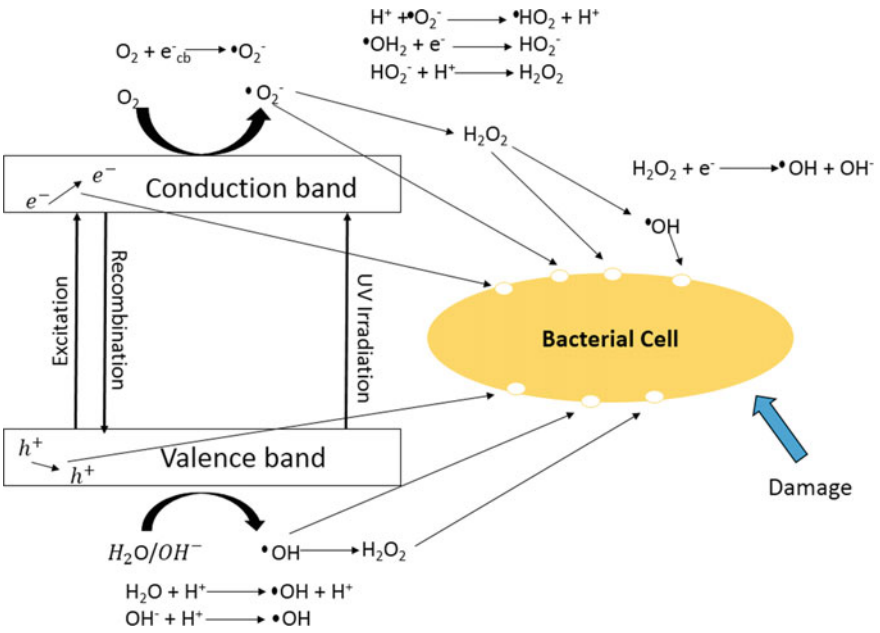

Fig. 6.6 Mechanism of disinfection when TiO_2 is used as photocatalyst under UV irradiation. Adopted from Wang et al. (2013)

showed that on UV irradiation it can be used effectively to reduce the time of disinfection, eliminating microorganisms from food that are pathogenic and to increase the food safety (Baruah and Dutta 2009). Though using TiO_2 has a major disadvantage, to activate the photocatalysis and initiate bactericidal action it requires UV irradiation. Nowadays, $Ag/AgBrTiO_2$ that are visible light absorbing photocatalyst have been proved effective in killing *E.coli* and *S.aureus* (Hu et al. 2006). Stoyanova et al. (2013) prepared TiO_2-ZnO nanocomposites by nonhydrolytic sol–gel method. Synthesized TiO_2-ZnO nanocomposites were found to be highly effective against *E. coli* in the presence of UV irradiation. In the case of visible irradiation only one log reduction achieved under 2 h. Wu et al. (2010) demonstrated the effect of Cu doped TiO_2 on *Mycobacterium smegmatis* and found that the growth rate of this bacteria reduced by three folds probably due to the release of Cu^{2+} ions from parent NPs. In *Salmonella typhimurium* TiO_2 was found to induce weak frameshift mutations (Pan et al. 2010) and also the NP was found toxic to *Psuedomonas aeruginosa* (Maness et al. 1999). TiO_2 nanoparticles can be used in dental applications because of its antibacterial properties against bacteria like *S. mutans* and *S. sanguinis* (Magraner et al. 2020).

Zinc Oxide Nanoparticles

Zinc oxide NPs among various other metal oxides that were studied have been found to be remarkably toxic. ZnO consisting of a band gap of ~3.2 eV similar to TiO_2 was treated as an alternative for photocatalytic activity against pathogens and other pollutants, such as pesticides and pharmaceuticals. ZnO had the same disadvantage as TiO_2, i.e. it is active only under UV irradiation. Its application as antimicrobials is favoured by its property of stability is harsh condition in addition to comparably low toxicity when conjugated with potent antimicrobials (Rai and Bai 2011). ZnO NPs have been found to be selectively toxic on bacteria with minimal side effects on cells of human body, these factors favour its recommendation to use it in food and agricultural industries. ZnO NPs have shown strong antimicrobial effect on food borne bacteria like *Salmonella typhimurium* and *Staphylococcus aureus.* Where ZnO NPs could cause complete lyse of these bacteria. ZnO NPs with 12 nm have been studied which showed inhibition of growth in *E. coli* by disruption of the cell membrane and by increasing the permeability of membrane. These finding support that the ZnO NPs can be effectively used in the applications in food industries to treat bacteria (King et al. 2018; Baruah and Dutta 2009). ZnO NPs were found to cause death by inducing oxidative stress and increasing cell permeability in methicillin-resistant *Streptococcus agalactiae* and *S.aureus* (Huang et al. 2008). Among various mechanism proposed to explain ZnO NPs antibacterial activity, the hydrogen peroxide generation from the ZnO surface is found to have potent action on inhibition of growth in bacteria. It is hypothesized, smaller size of particles and increase in number per unit volume increases the surface area and is responsible for generation of hydrogen peroxide. ZnO may also release Zn^{2+} ions which can be possibly another mechanism by which it can damage the cell membrane and interact with the intracellular contents (Brayner et al. 2006).

Studies also showed the effect of ZnO NP on methicillin-resistant *Staphylococcus aureus (*MRSA) and it was demonstrated that these NPs can get internalized inside cell that can cause cell membrane damage and disorganization of the cell wall. It is also known to increase oxidative stress inside the cell causing damage to the lipid, protein and DNA (Singh et al. 2014). Luo et al. (2013) showed the synergistic effect of ZnO with 25 different antibiotics against *S.aureus* and *E.coli.* They found that ZnO somehow enhanced the antimicrobial activity of penicillin, aminoglycosides, clarithromycin and tetracycline. ZnO NPs coated over glass surfaces were found to interfere with biofilm formation of *E.coli* and *S.aureus* (Applerot et al. 2012). Biocompatible nano-ZnO-bacterial cellulose (BC) has been found to show significant antibacterial activity against bacteria *B. subtilis* and *E. coli* (Dinca et al. 2020). Biogenic zinc oxide nanoparticles developed from aqueous *Pandanus odorifer* leaf extract (POLE) were found to show excellent antimicrobial activity against gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria *E.coli* (Hussain et al. 2019).

Gold Nanoparticles

The most important property of gold NPs is its biocompatibility which makes it an extensively used material in organisms. The biologically inert nature of gold NPs can be altered to have chemical functionality and also photothermal functionality. Gold nanoparticles, cages, nanorods and spheres on exposure to near-infrared radiation (NIR) showed destruction of cancer cells and killing of bacterial cells through photothermal heating. Combination of photodynamic antimicrobial chemotherapy with NIR photothermal radiation of Au nanorods that was conjugated with the photosensitizers killed methicillin-resistant *Staphylococcus aureus* (MRSA) (Rai and Bai 2011; Brown et al. 2012). Au NPs with light absorbing capacity combined with specific antibodies have shown to destroy Staphylococcus aureus with the help of laser. In case of Vancomycin Resistant *Enterococci* (VRE) it was found out that the effect of antibiotic vancomycin showed synergistic effect with gold NP coating (Wang et al. 2017). Cefaclor which is a second-generation β-lactam antibiotic, when used with Au-NPs showed higher effect on both types of bacteria *S.aureus* (gram positive) and *E.coli* (gram negative) as compared to when they are used alone. Peptidoglycan layer of cell wall becomes porous by the action of cefaclor which acts as a cell wall synthesis inhibitor. This action gets enhanced by the action of Au NPs which generated holes on cell wall, leading to cell leakage and death of bacteria. Possibility is also that gold NPs inhibit uncoiling of DNA and also its transcription via binding to it (Hajipour et al. 2012). In solution Au NPs produced Au^{3+} ions along with decarboxylation of citrate produced free radicals in presence of light and were found to be responsible for photomutagenicity in *Salmonella typhimurium* (Wang et al. 2011).

Magnesium Oxide Nanoparticles

Nanoparticle metal oxides that are highly ionic can be prepared having a high surface area in addition to unusual morphologies of the crystal that have numerous edge/corners as well as reactive surface sites. Aerogel procedure (AP-MgO) is used

for preparation of magnesium oxide (MgO) and MgO produced could be of varying shapes like polyhedral or square with size around 4 nm that is arranged within an extensive porous structure with ample pore volume (Rai and Bai 2011). AP-MgO NPs possess an interesting property for adsorbing and retaining elemental chlorine and bromine for a longer time (months), this combination of Ag-MgO/X_2 NPs can show potent killing activity against both types of bacteria and their spores. It can be used as a potent disinfectant. This property of Ag-MgO NPs to carry big amount of active halogens can be attributed to its high surface area and also enhanced surface reactivity (Pelgrift and Friedman 2013). The small size of NPs lets it cover around the cell of the bacteria to a large extent and this brings active halogens in high concentration in closeness to bacterial cell. These conditions in test against *Bacillus megaterium* and *E.coli* have shown good results and also against spores of Bacillus subtilis. AP-MgO/X_2 has a positive charge in water suspension, which is opposite to the charges on bacteria and spore cells increasing the effect of NPs and responsible for its bioactivity. It was observed using confocal microscopy that when the bacteria and NPs are together in water suspension, their opposite charges tend to bring them together in the form of aggregates. It was found that halogenated magnesium oxide has an active influence on bacteria and in particular their cell membranes. This was done using atomic force microscopy and electron microscopy studies. Hence, it was seen that the NPs of MgO with X_2 (Cl, Br) showed synergistic effects (both strong and fast) on bactericidal action and also more effective against the spores (Melander et al. 2018). Biofilm formation of *E.coli* and *S.aureus* was found to be inhibited with the use of MgF_2 NPs (Musee et al. 2011).

Aluminium Nanoparticles

Alumina NPs showed effect of growth inhibition on *E.coli* over a concentration range of 10–1000 μg/mL but this effect was observed only when the concentrations were very high. This can be associated with the surface–charge interaction of the particles with the cells. The prevention of cell wall disruption and desperate antimicrobial action is possibly because of its property of free-radical scavenging. Alumina has a corundum-like structure which is thermodynamically stable over a wide range of temperature. The corundum structure consisted of oxygen atoms with hexagonal close packing and two third of octahedral sites filled by Al^{3+} ions. Near neutral pH alumina NPs surface carried a positive charge. This resulted in adhesion of NPs to the negatively charged surface of *E.coli* which increased the concentration of NPs around it and negatively influenced its growth. The bacterial adhesion may be due to the electrostatic interaction of particle surface with bacterial surface in addition to the hydrophobic interaction as well as polymer bridging. Reactive oxygen species (ROS) generation can be causable factor for the antimicrobial effect of the metal oxides which disrupts the cell wall, finally cell death. But alumina as free radical scavengers have also been reported (Rai and Bai 2011). Al_2O_3 NPs of size 50–70 nm was found to cause damage to bacterial cell wall and increase its membrane permeability by binding to its cell wall in *E.coli* (Jiang et al. 2009).

6.3.3 *Factors Affecting Antibacterial Properties of Nanoparticles*

There are several factors that affect the nanoparticles (NPs) bactericidal properties, like size, shape, charge and others. Further environmental condition also has significant impact on its properties. It also depends on bacterial strain and the exposure time for its activity. The following factors are described briefly that affect the physicochemical properties of nanoparticles. Size of nanoparticle, smaller size provides it with large surface area that increases the probability of contact between bacteria and nanoparticles and help in passing through the cell membrane of bacteria compared to larger NPs. Shape was found that activity of NPs changed significantly with change in its shape with similar NPs. On increasing the roughness of NPs adsorption of bacterial proteins increases with the reduction in bacterial adhesion (Wang et al. 2017). Zeta potential has a significant influence on bacterial adhesion. The charges on NPs and bacterial cell membrane are important factors for the electrostatic force between them. Oppositely charged NPs tend to gather selectively at sites of bacterial infection and increase the vascular permeability. Doping modification was found to be one of the most adequate method by which we can regulate and have control over the NPs and bacterial interaction. Sahu and Biswas (2011) synthesized Cu-doped TiO_2 and found that with increasing Cu doping the crystal phase changed from anatase to rutile and the growth of particles were restricted and also the band gap came to visible region. Environmental conditions, the antimicrobial activities of NPs, were found to vary with different environmental conditions. The medium characteristics like pH and osmotic pressure influence the surface charge, aggregation and solubility of NPs which in turn affect its activity (Wang et al. 2017; Hajipour et al. 2012).

6.4 Conclusion and Future Perspectives

The bacterial strains have developed resistance against many generations of drugs, leaving very little options for treatment of bacterial infections. The gram-positive and gram negative-bacteria respond differently to the antibiotics due to the difference in their cell wall composition, and gram-negative bacteria tend to be more effected as compared to the gram-positive bacteria due to the difference in thickness and composition of cell wall. The mechanism of action of antibiotics on bacterial cell is mainly through three mechanisms, first by targeting its cell wall, second by inhibiting the protein synthesis or third by inhibiting the DNA replication of bacteria. The bacteria have also evolved various mechanisms to negate the effect of antibiotics on them, like increase in function of efflux pumps or changing antibiotic binding receptor sites. This change is brought about in bacteria either by genetical basis which includes mutational resistance and horizontal gene transfer both of which are inheritable to the next generation. Various kinds of NPs like Ag, Au, MgO, aluminium, TiO_2 and ZnO have antimicrobial action on different types of bacteria either individually or

in doped condition and generally follows mechanism of oxidative stress, dissolved metallic ions or non-oxidative stress conditions. Some NPs involve multiple mechanism simultaneously to kill bacteria. The antimicrobial action of NPs on bacteria are mainly through one of the following, firstly interaction with its cell barrier, either by creating pores in it or preventing it from formation or disrupting the cell barriers. The NPs penetrate the cell barrier either by diffusion or get adsorbed on the cell membrane. Secondly, by inhibiting the synthesis of bacterial protein and DNA. Third by regulating the expression of bacterial metabolic genes and also by inhibiting the formation of bacterial biofilm. The NPs advantage as antimicrobials can be summarized as it can be helpful in overcoming existing antibiotic resistance organisms, or it can combat microbes via multiple mechanism simultaneously or it can act as a good carrier of antibiotic. Several characteristics of NPs such as size, charge, shape, zeta potential, surface morphology and crystal structure and environmental conditions such as pH, osmotic pressure impact the antibacterial potential. With the desired characteristics, NPs can be utilized as an antimicrobial agent. Furthermore, NPs can employ several mechanisms simultaneously on bacteria and let it unlikely to develop resistance against it, thus having a potential of solving MDR in bacteria.

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