Review Article

Advances in Biogenic Nanoparticles and the Mechanisms of Antimicrobial Effects

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Innovations in the nanotechnological arena have paved a path leading to nano-revolution, which has most recently unfurled the role of plants in the biogenic synthesis of nanoparticles. Though synthesis of nanoparticles can be accomplished through physical and chemical techniques, biological course of synthesis has proficiently proved competent over other techniques. The problem of evolving multidrug resistant bacteria, due to irrational use of antibiotics, makes the biogenically synthesized nanoparticles attractive, due to their promising efficacy with negligible side effects. Consequently, the nanoparticles becoming better substitutes for conventional treatment besides overcoming all the limitations. Nanoparticles have great stability and potent antibacterial activity. The uniqueness lies in their size (10 and 500 nm) and dimension offers these particles to dynamically communicate with biomolecules on the cell surfaces and within the cells, so proficiently to decode and designate various biochemical and physiochemical properties of the cells. The present review aims to recapitulate various emerging efforts in the biogenic synthesis of nanoparticles, most significantly their unique mechanisms of action with different approaches as well as the factors that may add up to their antimicrobial activity.

Key words: Antibacterial, biocidal, biogenic, multidrug resistant, nanoparticles

In recent years, the ever-escalating use of high dosage of antibiotics has created the trauma of multidrug resistant (MDR) strains, particularly bacterial pathogens, thereby substantially mitigating the effect of concerned antibiotics. The reduced effectiveness of drugs testifies MDR bacteria could be a major threat to human health[1]. Therefore, the need to come up with a more effective and non-toxic treatment arose, which was followed by the advances in biotechnology pioneered the domain of nanotechnology. Nanomaterials (NM) are of great interest due to their novel physicochemical, magnetic and optoelectronic properties that are administered (metal NM are preferred topical administration route, while others like polymeric NM are administrated via oral as well as topical route) by their unique size, shape, and distribution (Table 1)[2,3].

"Nanotechnology is the application of science to control matter up to the molecular level"[4], and is currently one of the most active areas of research. Nanoparticles (NPs) are generally recognized as materials having at least one dimension between 1-100 nm[5,6]. It is considered as a transitional zone between individual molecules and the analogous bulk materials, which enable it to hold unique properties, which are peculiar from their molecular and bulk analogue[7,8]. There are many sources of NP synthesis, but the green approaches are gaining popularity. Plant parts such as, the leaf, the bark, the flower, the peel, and the seed and microorganisms such as fungi, bacteria, algae, yeast, actinomycetes, and enzymes offer clean, eco-friendly, non-toxic machinery for their synthesis, which is compatible with pharmaceutical and cosmeceutical applications[9-11]. These NMs have long been documented to exhibit microbiocidal, microbiostatic actions and serve as potential antibacterial agents in medical and industrial applications[12]. The highly reactive metal or metal oxide NP show bactericidal activity against both Gram-positive and Gram-negative bacteria[13]. The NPs have been known for targeted drug delivery; hence check specific microorganism growth. These properties have broadened their application

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to the genomics, biosensors, clinical chemistry and immune response enhancement[14]. This review aims to describe various approaches intended for biogenic synthesis of metal or metal oxide NPs, and to elaborate extracellular and intracellular biocidal mechanisms of these particles along with the factors that might influence the activity of NPs.

SYNTHESIS OF NPs

Various processes for the synthesis of NPs have been employed up to recently, mainly in the chemical, physical and biological field, but their fabrication was found to be expensive and the involvement of toxic chemicals for their synthesis makes the way for biological synthesis as a more preferred option. In biogenesis, bacterial, fungal and plant extract sources can be used for their synthesis; this type of fabrication is very reliable, cost effective and nontoxic[15]. Among all the preferred methods suggested for nanoparticle synthesis; the chemical reduction method and biological synthesis method were widely considered due to its advantage in controlling particle size and morphology very commendable. The comparative difference between chemically and biogenically synthesized NPs is shown in Table 1[15-17].

The two most commonly employed processes of NP synthesis involves the top down and bottom up pathway. In top-down pathway, the bulk materials are broken down to small particles of nanoscale by many lithographic methods like mechanical milling/ ball milling, and chemical etching. This approach introduces imperfections in the surface structure of the product, which become the major limitation since physical properties and surface chemistry of NPs are highly dependent on the surface structure[17]. In the bottom up pathway, the NPs are prepared from smaller strating materials through oxidation and bioreduction procedures (fig. 1). Atoms aggregate to form nuclei range at nanoscale, thus the NPs obtained are with negligible flaws. The biological procedure involves capping and stabilizing mediators (phytochemicals like phenolics, flavonoids, terpenoids, and cofactors) that contribute higher stability[17,18] (fig. 2). Apart from this, advanced studies reflected the succeeding role of microorganisms in green synthesis because of the ease of process involved in the synthesis, much stable NPs and cost effectiveness[19,20]. The biogenic synthesis uses varied life forms, starting from the very simple prokaryotic bacterial cells to composite eukaryotes such as angiosperms[21]. Various green sources employed for biogenic synthesis and their impact is summarized below.

Bacterium-mediated green synthesis:

The ability of bacteria to produce inorganic intracellular and extracellular materials makes them prospective biofermenters for the synthesis of gold and silver NPs (AgNPs). Silver is well-known for its biocidal activity, however, there are bacteria that are resistant to silver[22] and can facilitate the cell to accumulate silver on its cell wall up to 25 % of their dry weight biomass. Therefore, initiating the role of these bacteria (Table 2) in industries for the recovery of silver from ores[23]. *Pseudomonas stutzeri* AG259 (first silver resistant bacteria) was used for the synthesis of noble NPs. Bacteria cultured in high concentration of silver nitrate can accumulate silver in large bulk (with nanoscale 200 nm diameter)[24]. *Proteus mirabilis* PTCC 1710 found even more proficient for NPs synthesis[25]. Further, it was reported that during incubation of bacteria the type of broth (nutrient broth, Muller-Hinton broth) used may promote synthesis of NPs (extracellular or intracellular). Therefore, selected and controlled incubation creates bacteria-based

TABLE 1: COMPARATIVE DIFFERENCES BETWEEN BIOGENIC AND CHEMICALLY SYNTHESIZED NANOPARTICLES[5,9,11,13,15-17]

Properties	Chemical	Biological
Nature	Expensive, toxic	Cost effective, nontoxic
Reducing agent	Dimethylformamide, ethylene glycol, hydrazine hydrate, sodium borohydride, polyol, sodium citrate and N,N-dimethyformamide	Biomolecules include phenolics, polysaccharides, flavones, terpenoids, alkaloids, proteins, amino acids, enzymes, predominantly, nitrate reductase
Method	Stabiliser (surfactant) is added to the first solution to prevent the agglomeration of nanoparticles	There is no need to add a stabilising agent
Environmental impact	Environmental pollution is a disadvantage of the chemical method and the chemical reduction methods are energy-intensive	Synthesis carried out in environmental conditions and they are safe enough, and consume no energy
Antibacterial activity	The chemically synthesized nanoparticles showing comparatively lower antimicrobial activity against pathogenic bacteria	The nanoparticles synthesised from biological means are showing better antimicrobial activity against the pathogenic bacteria

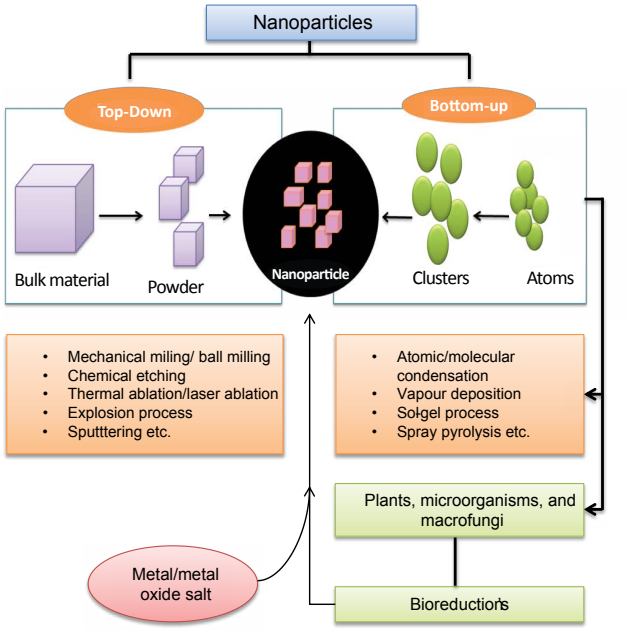

Fig. 1: Schematic representation of protocol employed for synthesis of nanoparticles[41]

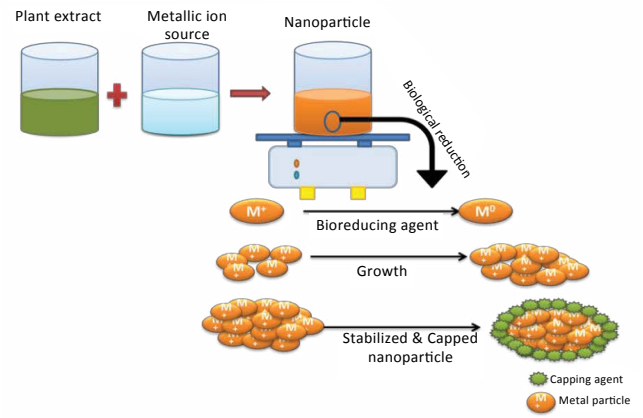

Fig. 2: Schematic representation of mechanism of biological synthesis of nanoparticles using plant extracts

synthesis of NPs more flexible, cost effective and it is the appropriate method for large scale production[26]. However, there is a drawback regarding bacterial nanofactories, firstly rate of synthesis is slow, available size and shapes are limited as compared to the conventional process of NPs synthesis. Hence, other biogenic procedures were investigated[27].

Fungus-mediated synthesis:

Tolerance to bioaccumulation of metals, high binding ability and intercellular uptake makes fungi more efficient for biological synthesis of NMs[28]. The protocol for the synthesis of NPs is diverse: the enzymes secreted by fungus are efficient in silver ion reduction and induce metal NP production[29]. This method had been practiced in the beginning of 20th century, using *Verticillium* fungus for the production of AgNPs with diameter of 25±12 nm and spherical morphology[30,21]. In contrast to bacteria, NPs were formed below the fungal cell[31] whereas, further investigations reported diverse morphologies within range from spherical, triangular to hexagonal[24]. The mechanisms of production of NPs hypothesized were, NPs are formed on the surface of mycelia instead of in solution. Due to electrostatic interaction between negatively charged carboxylate groups in enzymes (present in the cell wall of mycelia) and positively charged silver ions, the silver ions get adsorbed on surface of the fungal cell. Then the fungal enzyme reduces silver ions to silver nuclei[32]. Fungal synthesis of NPs offers advantages of carrying simpler bottom-up processing and easy handling of biomass.

The use of fungi in the synthesis of NPs is significant as the fugal cell secretes much higher amount of protein than bacteria that enhance the green production of NPs significantly, hence employed for large scale production of metal NPs. The NPs are produced extracellularly, therefore they are easy to purify and can be directly used in various applications[33]. The fungal mycelia mesh can tolerate flow pressure and other conditions in bioreactors as compared to plant material or bacteria[34]. Most fungi have a high tolerance towards metals and have high wall-binding capability, as well as intracellular metal uptake capabilities. Therefore, natural nanofactories shifted from bacteria to fungi. For instance, the white rot fungus (*Phaenerochaete chrysosporium*) is nonpathogenic and this contributes to the large scale production AgNPs[34]. Apart from this, the fungal green synthesis offers rapid synthesis. *Aspergillus fumigatus* facilitate obtaining monodispersed AgNPs within matters of minutes[35]. A. flavus NPs combined with antibiotics enhance the antibacterial activity against the MDR bacteria[36].

Yeast-mediated green synthesis:

Yeast was also examined for silver NP green synthesis[37,38]. Some yeast strains (MKY3) are silver tolerant, initially, that were used for extracellular synthesis (Table 2)[39-45]. Recently, the biosynthesis of gold and AgNPs was investigated using the culture supernatant broth of the yeast *Saccharomyces cerevisae* and *Candida guilliermondii*. Metal NPs were formed after gold and silver ions came in contact with the culture supernatant broth[46].

Plant-mediated green synthesis:

Green synthesis of NPs using plant extracts is advantageous as plants are easily accessible, clean,

ecofriendly, safe to handle with the variety of highly active constituents that initiate the reduction of metal ions. The first approach toward plant-mediated synthesis of NPs was made with Alfalfa sprouts[47]. The root of Alfalfa can absorb silver from the surrounding growing medium (i.e. agar) and transfer it to the shoots in the same oxidation state. The translocated silver in the shoots is accumulated and arranged in such a way to form NPs. The production of NPs with plants gives faster rate of synthesis than bacteria and fungi. *Geranium* leaf extracts take about 9 h to reach 90 % completion of NPs synthesis compared to 24 to 124 h required for other reactions stated previously[48]. Since then, the use of plant extracts in the synthesis of NPs escalated leading to a new era in green synthesis. Many investigators have demonstrated that the synthesis of NPs by plant extracts can be accomplished in the metal salt solution within a matter of minutes at room temperature, however, rate of synthesis depends on the nature of plant extracts (Table 3)[49-60]. There are many other factors as type of metal salt used, concentration of plant extract, temperature and pH, which affect the synthesis of NPs[26].

Almost all parts of plants such as leaves, root, stem, latex, flowers and seeds have been employed for NP synthesis[61]. The biomolecules present in plants act as both reducing agents as well as capping agents that stabilize and govern the morphology of NPs (fig. 2). The leading biomolecules that involve as bio-reducing and capping agents of the metal NPs include, phenols, polysaccharides, flavones, terpenoids, alkaloids, proteins, amino acids, enzymes and alcoholic compounds. However, there are reports that chlorophyll pigments, quinol, methyl chavicol, linalool, eugenol, caffeine, ascorbic acid, theophylline and other vitamins also reduce the metallic salt to NPs[62-67]. The aforementioned phytochemicals have ability of reducing, stabilizing, capping and preventing accumulation of NPs. The hydroxyl and carboxyl group of phenolic compounds bind to the surface of metals[68].

ANTIMICROBIAL ACTIVITY OF NPs

The ever escalating popularity and applications of NPs have impacted every possible field of research and scientific publication. The biogenically synthesized NPs also referred to as the green generation of NPs, the applications of which have resulted in sustainable advances in medicines, diagnosis and clinical applications[69-71]. The use of NPs is considered as a substitute for antibiotics with better efficacy with negligible side effects and for combating bacterial multidrug resistance. The green synthesized metal or metal oxide NPs have potent antibacterial, antifungal as well as antiparasitic activities[72-76]. The toxicity of metal NPs can be analyzed by various *in vitro* and *in vivo* studies. These studies reveal that NP-induced toxicity of metal-based NPs can affect the biological behaviour of the organ, tissue, cellular, subcellular and protein levels. The unique size of the NPs allows it to easily penetrate the cell and cause adverse effects (fig. 3). It is evident that metal-based NPs due to their biological and physiochemical properties are promising as antimicrobials and therapeutic agents (Table 4)[77-79].

NPs, in particular have tremendous biocidal effects[71,80] and are therefore fascinating in a scientific field, especially for the production of new class of antimicrobials[81]. Though antimicrobial activity of AgNPs is of wide spectrum, the morphologically and metabolically different microorganisms appear to associate with multidimensional mechanisms of NPs to interact with microbes[82]. The structure, shape, and size of NPs and their mode of interaction with the surface of microbes offer a distinctive mechanisms of impairment and distinctive level of biocidal effects (fig. 3). The bactericidal efficacy of the NPs is influenced by concentration, size and shape of NPs and the studied microrganisims[83,84]. Small sized NPs seems to have a high tendency to penetrate deeper into the bacterial cell wall, and interact with the membrane leading to cell membrane damage. Certainly, the bactericidal excellence is much dominant in the smaller size of NPs than larger size with positive zeta potential. The NPs with zeta potential create electrostatic forces with the bacterial cell in contiguous. It is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. As the bacterial cell wall has negative charge, promotes attraction between the two entities and hence penetration in to the bacterial membrane (fig. 3).

MECHANISM OF ANTIMICROBIAL ACTIVITY OF NPs

These include, direct uptake of NPs, indirect activity of NPs by production of reactive oxygen species (ROS) and impairment of cell wall/membrane.

Direct uptake of NPs:

As previously mentioned, antimicrobial properties of silver are remarkable; thus, it is probable that eluted

TABLE 2: BIOLOGICAL ENTITIES USED IN SYNTHESIS OF METAL AND METAL OXIDE NPs WITH THEIR SIZE, SHAPE AND BRIEF BIOCIDAL ACTIVITY[84-90]

Biogenic origin	NPs	Morphology	tBiocidal effects	Ref.
Bacteria				
Pseudomonas stutzeri AG259	Ag	200 nm/various shape	Deal with the metal toxicity stress in the environment	23
Proteus mirabilis PTCC1710	Au	10-20 nm/spherical	-	25
Escherichia coli	CdS	2-5 nm/spherical	Used to synthesize green solar cell and effective against *E. coli* (BW25113)	83
Bacillus mycoides	TiO2	40-60 nm/spherical	Suppress aquatic biofilm growth	84
Bacillus subtilis	TiO2	10-30 nm/spherical	Bioremediation without producing toxic chemicals to the environment	85
Bacteria strains NS2 and NS6	PbS	40-70 nm	Bioremediation without producing toxic chemicals to the environment	86
Aeromonas hydrophila	ZnO	57-72 nm/spherical	Exhibited antimicrobial activity against both bacteria (*Pseudomonas aeruginosa*) and fungi (*Aspergillus flavus*)	53
Fungus				
Aspergillus flavus	TiO2	62-74 nm/oval	Effective against *S. aureus*	87
Colletotrichum sp.	Au	20-40 nm/spherical	-	88
Fusarium oxysporum	Au	20-40 nm/spherical, triangle	Antibacterial activity against burns bacterial growth, *E. coli*, *S. aureus*	16
Volvariella volvacea	Ag & Au	20-150 nm/spherical/ hexagonal	Shows antimicrobial activity	89
Verticillium sp.	Ag	25±12 nm/spherical	Shows antimicrobial activity	30
Yeast				
MKY3	Ag	2-5/hexagonal	Activity against *E.coli*, *S aureus*	37
Schizosacchromyces pombe	Cd	1-2/hexagonal	-	90

TABLE 3: PLANTS/PLANT PART-MEDIATED SYNTHESIS OF METAL AND METAL OXIDE NPs WITH THEIR SIZE, SHAPE AND BRIEF BIOCIDAL ACTIVITY[91-102]

Biogenic origin	NPs	Morphology	Biocidal effects	Ref.
Alfalfa	Ag	2-20 nm	Significantly increases root & stem growth, antibacterial	39
Avena sativa	Au	5-20 nm/rod shaped	-	18
Aloe vera	Au and Ag	50-350 nm/spherical, triangular	Bactericidal effects	91, 92
Camellia sinensis	ZnO	30-40 nm/spherical, triangular	Strong antimicrobial effects	93
Catharanthus roseus	TiO_2	25-110 nm/irregular	Effective again *Hippobosca maculata* (flies) and *Bovicola ovis* (lice)	94
Cassia auriculata	ZnO	-	Used as an effective stabilizing, reducing agent for the synthesis of NPs	87
Cassia alata	CuO	110-280 nm/spherical	Application in medicine	50
Croton sparsiflorus	Ag and Pd	22-52 nm/spherical	Shows biocidal effects against *S. aureus*, *E coli*, *B. subtilis*	95
Euphorbia condylocarpa	Fe_3O_4	Avg. 39 nm	Magnetically recoverable and recyclable catalyst	96
Emblica officinalis	Ag and Au	20-25 nm/spherical	Antibacterial activity	96
Euphorbia hirta	Ag	50 nm/spherical	Biocidal activity	97
Gloriosa superba	CuO	5-10 nm/spherical	Effective against *S. aureus* and *Klebsiella aerogenes*	98
Gum karaya	CuO	Avg. 4.8 nm 16-	Antimicrobial activity against *E. coli*	99

Geranium leaves	Ag	40 nm/quasilinear Superstructure	Antimicrobial activity	39
Hibiscus rosa sinensis	Ag and Au	14 nm/spherical	Strong antimicrobial activity	93
Ipomoea aquatica	Ag	Prism 100-400 nm/ spherical, cubic	-	101
Jatropha curcas	Ag	15-50 nm/spherical	Biocidal effects	102
Malva sylvestris	CuO	5-30 nm/spherical	Effective against both Gram +ve and -ve bacteria	55
Phyllanthus amarus	CuO	20 nm/spherical	Effective than rifampicin against *B. subtilis*	100

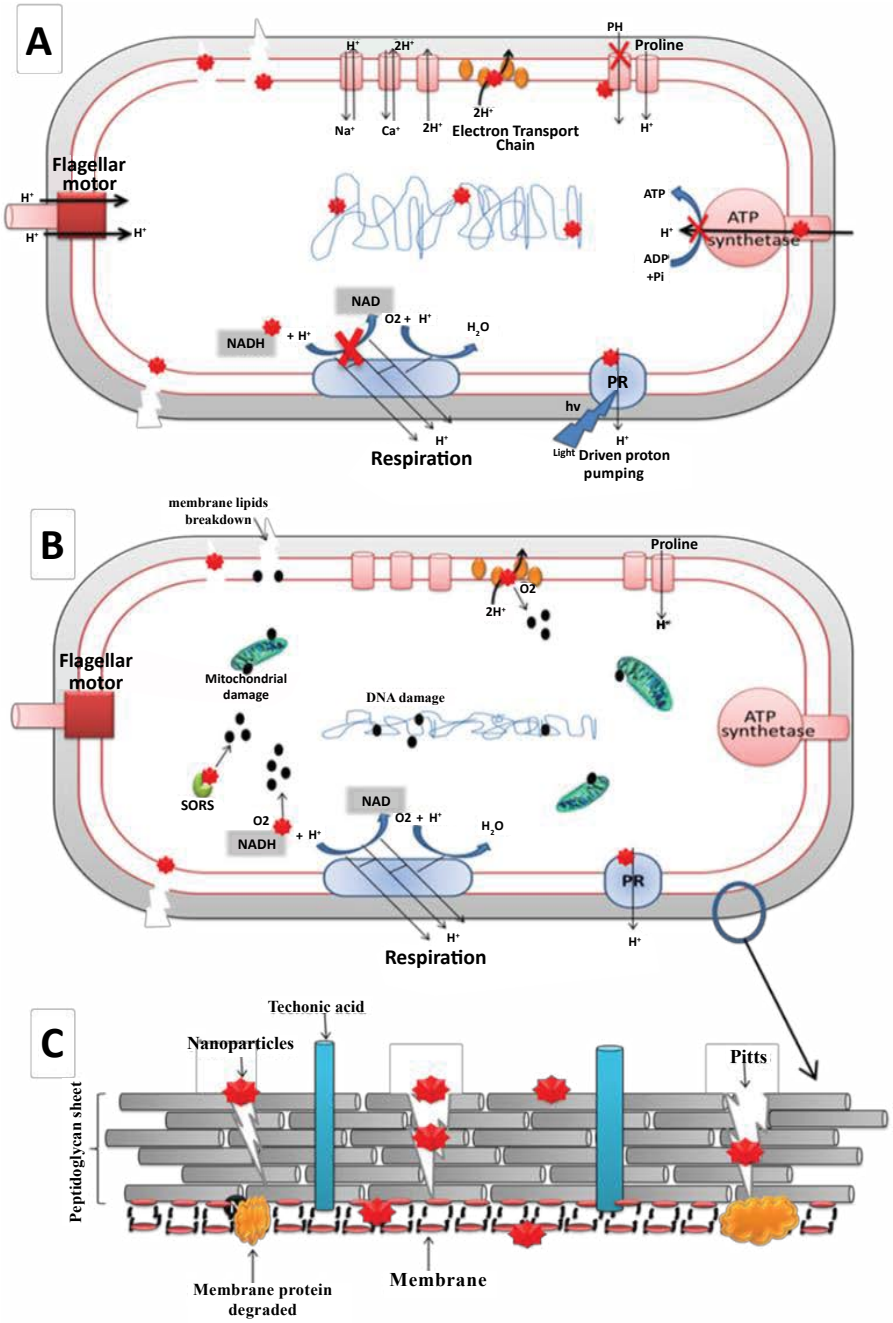

Fig. 3: Schematic representation of biocidal effects of nanoparticles on bacterial cell

(A) Cell uptake of Ag+ directly, Ag+ interacts with NADH dehydrogenase (respiratory chain enzyme), leads to uncoupling of respiration from ATP synthesis, Ag+ binds with transport protein, consequently proton leaks out, that collapse the proton motive force, efflux of intracellular phosphate; (B) nano activate ROS sault membrane lipids, abolishing the respiratory chain enzymes by direct interactions with thiol groups in these enzymes or the superoxide radical scavenging enzymes such as superoxide dismutases, mitochondrial and DNA damage occurs; (C) NPs leads to the pits formation in membrane that variates the permeability of the cell membrane due to release of lipopolysaccharides and proteins of membranes

TABLE 4: COMMERCIALLY AVAILABLE NPs COMPOSED OF METAL/METAL OXIDE IONS USED FOR ANTIMICROBIAL ACTIVITY[103-105]

Metallic constituents	Nanoparticles	Antibacterial activity	Reference
Pure metal	Silver	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus subtilis*	39, 92, 95
	Gold	*Corynebacterium pseudotuberculosis*, *K. pneumoniae*, *S. typhi*, *P. aeruginosa* and *E. coli*	18, 92, 96
Single-metal oxide	Aluminum oxide	*E. coli* DH5α, *S. epidermidis*, *Scenedesmus* sp. and *Chlorella* sp.	
	Copper oxide	*E. coli*, *P. aeruginosa*, *K. pneumonia*, *Enterococcus faecalis*, *Shigella flexneri*, *Salmonella typhimurium*, *P. vulgaris*, and *S. aureus*	55, 100 98, 99
	Silicon oxide	*E. coli*, *S. aureus*, *Bacillus*, *P. aeruginosa*	103
	Titanium oxide	*P. aeruginosa*, *E. coli*, *S. aureus*, *E. faecalis*, *Pichia jadinii*, *Hippobosca maculata*, *Bovicola ovis*	94
	Zinc oxide	*E. coli*, *K. pneumoniae*, *S. dysenteriae*, *S. typhi*, *P. aeruginosa*, *B. subtilis* and *S. aureus*	87, 93
Multi-metal oxide	Copper-substituted cobalt ferrite	*E. coli*	104
	Indium tin oxide	*E. coli*, *S. aureus*	105

ions from AgNPs are responsible for their antibacterial properties. Ag+ has high affinity to thiol groups in cysteine residue of respiratory and transport protein[85]. Therefore, at sub-micromolar concentrations cells take up Ag+ directly, where it interacts with enzymes of the respiratory chain (NADH dehydrogenase) subsequently uncoupling of respiration from adenosine triphosphate (ATP) synthesis occurs. Ag+ also binds with transport protein consequently proton leaks out, stimulating collapse of the proton motive force[85,86]. Ag+ obstructs the uptake of phosphate and thus initiates the efflux of intracellular phosphate[87]. Further, it is reported that Ag+ increases DNA mutation frequencies during polymerase chain reactions[88]. Bacterial cells exposed to millimolar Ag+ doses endure morphological modifications such as DNA compression and localization in an electron-light region in the core of the cell, cytoplasm retrenchment, and degeneration of cell wall/membrane allowing leakage of intracellular contents[89]. Hence, physiological as well as morphological changes occur (fig. 3).

The indirect activity of NPs through production of ROS:

ROS are prospective by-products of the metabolic pathway of respiring organisms. Although antioxidant defence of the cell (glutathione/glutathione disulfide GSH/GSSG ratio) guards the cells to some extent from ROS, excess ROS production may produce oxidative stress[8] and can assault membrane lipids, consequently, leads to impairment of membrane and mitochondrial dysfunction and DNA damage[90]. Metals can act as catalysts and generate ROS in the presence of dissolved oxygen[91]. Therefore, AgNPs may catalyse the reaction with oxygen directing to release excess free radicals. In bacterial cells, Ag+ are probably to induce the generation of ROS by abolishing the respiratory chain enzymes by direct interactions with thiol groups in these enzymes or the superoxide radical scavenging enzymes such as superoxide dismutases (fig. 3)[92]. The study by Kim *et al*. confers the presence of free radicals from AgNPs by means of spin resonance measurements[82]. They observed that the toxicity of AgNPs and silver nitrate was eliminated in the presence of an antioxidant, approving antimicrobial mechanisms of AgNPs against *Staphylococcus aureus* and *Escherichia coli* was interrelated to the formation of free radicals from the surface of AgNPs and consequent free radical persuade membrane damage.

Impairment of cell wall:

The unique size of the NPs allows it to easily penetrate inside the bacterial cell. Studies show that AgNPs adhere to and penetrate within *E. coli* cells at sizes much smaller than the original particles; moreover[93,94] leads to the pits formation in membrane that variates the permeability of the cell membrane due to release of lipopolysaccharides and proteins of membranes[95] (fig. 3). AgNPs degrade the peptidoglycan structure, and cell wall destruction proved by release of

muramic acid, reported in *S. aureus*. Further, the gas chromatography-mass spectrometry (GC-MS) analysis (tandem) and muramic acid release validate the probable decomposition of glycan strands. These reports prove that AgNPs hinged with both the sides of peptidoglycan stratum of bacterial cell wall. Silver binds to beta-1,4- bonds of N-acetylmuramic acid and N-acetylglucosamine of peptidoglycan strands, and degrading their bond hence muramic acid set free[96] (fig. 3).

The interaction between NPs and bacterial cells are due to electrostatic attraction between negative charges on the cell membranes and positive charge of NPs. However, this mechanism is inefficient to explain the adhesion and uptake of negatively charged AgNPs. It is also hypothesized that the preferential sites of uptake and interaction for AgNPs and membrane cells might be sulphur containing proteins in a similar way as silver interacts with thiol groups of respiratory chain proteins and transport proteins, interrupt their proper functioning[97]. Proteomic data demonstrate the accumulation of envelope protein precursors in *E. coli* cells after exposure to AgNPs. Energy from ATP and proton motive force is required in order to synthesize envelope proteins and to translocate it to the membrane; therefore cytoplasmic accumulation of protein precursors suggests degeneracy of proton motive force and depletion of intracellular levels of ATP.

FACTORS AFFECTING THE ANTIMICROBIAL ACTIVITY OF NPs

Concentration and size:

The influence of size and concentration has been analyzed in many NPs. AgNPs with different sizes at a low concentration of 0.01 ppm have been evaluated for activity[82]. The smallest-sized spherical AgNPs were more efficient to kill and destroy bacteria as compared to larger spherical AgNPs. Due to the high surface to volume ratio, the smaller-sized NPs released more silver cations and thus, proved more effective to kill the bacteria as compared to larger-sized particles[98].

Chemical composition:

The chemical composition is the base of the NPs that decides the variations in their activities. The NPs are recognized to produce ROS (TiO_2, ZnO_2 and SiO_2) against *Bacillus subtilis* and *E. coli*. The biocidal activity of these compound was found in ascending order from SiO_2 to TiO_2 to ZnO. The growth of *B. subtilis* was 90 % inhibited by 10 ppm concentration of ZnO NPs, while growth of *B. subtilis* effectively inhibits to 90 % by 1000 and 2000 ppm of TiO_2 and SiO_2, respectively. Whereas, the inhibition effect of NPs on *E. coli* was partially at 10 ppm of ZnO NPs and 500 ppm of both the NPs[99]. Further, it was specified that the bactericidal activity does not effects by the light or dark, suggesting growth inhibition involves mechanisms other than ROS production.

The shape of NP:

Studies conducted regarding the shape of the NPs have suggested that different shapes (spherical, elongated rod and truncated triangular) of AgNPs have different intensity of biocidal activity. The media supplemented with different shapes of NPs have different colony-forming unit count of *E.coli*. The activity of NPs seems to get stimulated by the morphology of NPs. The shape-dependent activity was determine in the term of facets, the spherical NPs primarily had 100 facets, rod-shaped NPs had 111 facets on side surface and 100 on end, truncated triangular NPs with 111 facets on top basal planes. The facets 111 are of high atom density that favors antibacterial reactivity of NPs[100].

Target microorganisms:

Many studies have reported that NPs showed greater biocidal activity against Gram-negative rod-shaped bacteria than Gram-positive cocci. The effect of AgNPs was analyzed using *E. coli* and *S. aureus*, where results showed significantly more activity against *E. coli* (MIC 3.3-3.6) than *S. aureus* (MIC more than 33 nM). The difference in results depicted difference in the cell wall organization, as the cell wall composition of Gram-positive bacteria (*S. aureus*) consist of higher concentration of peptidogylcan[101]. However, Huang *et al*. revealed the activity of ZnO NPs against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria[99]. Whereas another study shows ZnO NPs higher activity against *S. aureus* than *E. coli* and *P. aeruginosa*[102].

Photo activation:

The NPs of TiO_2 showed activity against *E. coli*, and this activity significantly increases with UV radiations[20]. Further, it was reported that TiO_2 NPs has negligible activity i.e. it shows about 20 % of growth inhibition of *S. aureus* without photoactivation. On the other hand, ZnONPs show escalating activity after photoactivation

by UV radiation including visible light[103,104]. Lipovsky *et al*. has demonstrated that the photoactivation with blue light of these NPs enhances their activity by enhancing ROS production, with prominent effect in ZnO NPs[105]. Although, one can not be oblivious to the fact that bacterial incubation with NPs in dark condition has no effect on surviving of microorganisms[99].

Isolation and purification of NPs

After centrifugation of metal NPs solution for 10 min at 10 000 rpm, the NPs are settled at the bottom of the conical tube. The supernatant phase is removed and NPs is used to wash with 10 ml water for three times. After the washing, the residue is transferred to freeze dryer.

Depending upon the preparation method used, various other impurities can be found in the NPs suspensions. Simple filtration will only remove polymer aggregates, while other impurities require more sophisticated procedure. The most common procedures are gel filtration, dialysis and ultracentrifugation. However, these methods are not entirely satisfactory because they are not capable of removing molecules with high molecular weights. This needs to development of cross flow filtration method in which the NPs suspension is filtered through membranes. The suspension is passed via several filtration cycles while filtrate containing components smaller than pores is discarded.

Recently, the use of microorganisms and plants for NP production has proved to be quite efficient. Simple bacteria to complex eukaryotes have been employed for the synthesis of NPs of desired size and shape. The green synthesis proved to be sustainable, eco-friendly, stable, non-toxic and cost effective. The production of green NPs can be equipped on large scale, with non-toxic plant easy disposed of. Most green synthesis approaches represent promising alternative approaches to antibiotics particularly dedicated to Ag and Au NPs. Other biogenically synthesized metals and its oxides NPs have commanding roles in human welfare. However, attention should be drifted toward the activity of NPs in combinations with antimicrobials of other class against MDR microorganisms. These can be used to address a number of challenges in the field of nanomedicine. But it must be remembered that they can also possibly cause adverse biological effects at the cellular and subcellular levels. Therefore, after the cytotoxicity and clinical studies, the NPs can find immense application as antimicrobials in the consumer and industrial products. Another aspect that is worth consideration is the factor that may influence the activity, shape, and size of NPs for enhanced production. Many studies illustrated the significant deviation in chemical composition of plant of same species when collected from habitats of different regions and thus lead to variation in the results.

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Conflict of interest:

All authors declare that they have no conflict of interests.

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