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Nanobiotechnological Approaches Against Multidrug Resistant Bacterial Pathogens: An Update

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Abstract: Multiple drug resistant bacteria remain the greatest challenge in public health care. Globally, infections produced by such resistant strains are on the rise. Recent advent of genetic tolerance to antibiotics in many pathogens such as multiple drug resistant *Staphylococcus aureus* is a matter of concern, prompting researchers and pharmaceutical companies to search for new molecules and unconventional antibacterial agents. Recent advances in nanotechnology offer new opportunities to develop formulations based on metallic nanoparticles with different shapes and sizes and variable antimicrobial properties. This article is an extensive literature review that covers the latest approaches in the development of new and unconventional antibacterial agents using nanobiotechnological approaches which will better equip scientists and clinicians to face the challenges in view of dwindling stocks of effective and potent antimicrobial agents and formulations.

Keywords: Antimicrobial drugs, multiple drug resistant bacteria, nanoparticles, nanotechnology, *Staphylococcus aureus*.

BACKGROUND

The worldwide emergence and re-emergence of drug resistant pathogens pose a grave challenge especially in the field of biomedicine and pharmaceuticals. Multidrug resistant (MDR) pathogens and parasites pose a great challenge as an individual infected with such strains has to suffer a lot due to difficulties in diagnosis, investigation, treatment, lengthier hospital stay and also due to side effects of multidrug regimens [1, 2], prompting researchers to increase the bactericidal potential by developing new or modifying the available compounds [3]. In view of challenges faced and a paradigm shift in the approach of researchers, nowadays, nanotechnology provides a platform to modify and develop nanoparticles (NPs) which can act as promising tools in various fields like diagnostics, as biomarkers, antimicrobial agents, for cell labeling, drug delivery systems, contrast agents and nano drugs which can be used as disinfectants or antiseptics [4-8].

It is no wonder that nanotechnology is fast emerging as a field with enormous opportunities in various practices [9]. The NPs of metals like silver (Ag), gold (Au) and platinum (Pt) are extensively used as antimicrobials, as agents in gene and drug delivery as well as in diagnostic sensors [10]. Nanotechnology also offers hope to develop environmental and health friendly nanomaterials that would replace the current physiochemical processes associated with hazardous by-products [11, 12]. Moreover, in view of growing antibiotic resistance, alternative strategies are also being researched and encouraged [13].

The efficacy of distinct nanoconjugates against drug resistant pathogenic microorganisms in the recent past has also encouraged the use of nanomedicine and prompted scientists to develop newer ways to counter the drug resistance. One such approach is the exclusive utilization of metal composites at the nano scale. Certain enzymes and genetic sequence mutations may disrupt MDR mechanisms by altering the medicine efflux from the cells [14]. Advances in the field of nanotechnology to counter MDR bacteria by using NPs are also being applied by microbiologists and clinicians worldwide to control infections [15-18]. Simple explanation for the recent rise of NPs as an alternative to control MDR bacteria could be their large surface to volume ratios and crystallographic surface structure which effectively covers the microorganisms and reduces oxygen supply for respiration [18, 19].

NANOTECHNOLOGY IN THE AREA OF MEDICINE

Nanomedicine refers to the implementation of nanotechnology for monitoring, diagnosis and treatment of diseases, which appears to be a relatively recent trend but the association dates back to the use of lipid vesicles in 1965, which later became known as liposomes, the initial from history include restrained discharge polymer system of macromolecules in 1976, elongated circulating stealth polymeric NP in 1994, quantum dot bioconjugate in 1998 and nanowire nanosensor in 2001 [20-25]. Recent insights on the use of targeted NP contrast agents for initial characterization of cardiovascular pathology and atherosclerosis, both at molecular and cellular levels, appears to open new frontiers for effectively connecting imaging and normal drug delivery [26]. The use of markers based on nanotechnology will help in early/precise diagnosis, efficient therapy monitoring, and better patient management, thereby improving patients' condition of life and lowering fatality rates [6, 27, 28].

HEALTH AND SAFETY IMPLICATIONS FROM NPs

Nanopollution is one of the areas of concern that occurs as a result of the byproducts generated by nanodevices and manufacture of nanomaterials. Nanodevices and nanomaterials pose great health

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and environmental hazards owing to their small sizes, ability to float in air and greater penetrative powers.

Toxicity of nanomaterials is proven but the exact mechanism appears to be unknown. One hypothesis attributes the toxicity of nanomaterials to the "oxidant-antioxidant imbalance" leading to oxidative stress [29], production of inflammatory cytokines and cell death [30]. Since smaller sized nanomaterials may be easily consumed by cell organelles like mitochondria [31], nucleus [32, 33], they have the potential to cause DNA mutations, major structural damage and cell death [34]. Toxicity depends upon various factors like chemical composition, size, shape, aggregation, surface structure, charge and solubility of the nanomaterials [35].

SILVER NANOPARTICLES (AgNPs) EFFECT ON THE PHOSPHOTYROSINE PROFILE OF BACTERIAL PROTEINS

By using specific monoclonal antibodies to examine the phosphotyrosine substance of proteins derived from Gram-negative and Gram-positive bacteria, the possible effect of NPs on signal transduction affecting growth can be determined. Phosphorylation of different protein substrates is currently full-fledged and observed to affect bacterial signal transduction [36, 37]. AgNPs and NPs have shown little effect on tyrosine phosphorylated proteins in *Staphylococcus aureus* but, in contrast, notable dephosphorylation of two peptides of comparative masses 150 and 110 kDa has been observed in *Salmonella typhi* and *Escherichia coli* [38]. Decreased phosphorylation may imitate inhibition of the activity of different protein substrates such as UDP glucose dehydrogenases and RNA polymerase sigma factors with vital implications on bacterial growth [39]. Recently, tyrosine phosphorylation of bacterial single stranded DNA-binding proteins (BsSSB), that is, the phosphosignalling pathway important for cell cycle progression of bacteria has been described [40, 41]. Phosphorylation of protein tyrosine kinases implicated in capsular polysaccharide biosynthesis, exopolysaccharide and transport has also been studied in different Gram-positive and Gram-negative bacteria [42].

AgNPs AS ANTIBACTERIAL AGENTS

Silver nanoparticles have broad spectrum antibacterial actions and are also non-toxic to humans at low concentrations [43]. Silver ions (Ag^+) and Ag-based compounds are toxic to various pathogens including MDR *Pseudomonas aeruginosa* as well as methicillin-resistant *S. aureus* (MRSA), erythromycin-resistant *Streptococcus pyogenes* and ampicillin-resistant *E. coli* O157:H7 [44-47]. Thus, AgNPs can be used effectively against drug-resistant pathogens [48]. Effective antimicrobial surface coating agent properties are demonstrated by hybrids of AgNPs with amphiphilic hyperbranched macromolecules [49].

Various properties of AgNPs such as concentration, size and the sensitivity of the microbial species appear to affect the molecular processes within microbes, resulting in variety of effects which range from loss of infectivity, inhibition of growth and even cell death but the exact and precise mechanism remains unknown [50-55]. Various other mechanisms reported are electrostatic attraction between the positively charged NPs like AgNPs and the negatively charged cell membrane of the microorganism, association between concentration of AgNPs and formation of pits in the positively charged bacterial cell wall [56, 46], with subsequent accumulation and disturbances in the permeability of membrane, resulting in cell death. Progressive discharge of membrane proteins and lipopolysaccharide molecules due to production of pits in the membrane permeability and outer membrane change provide one possible explanation for the antimicrobial mechanism of AgNPs [57]. An identical mechanism causing the deterioration of the *E. coli* membrane structure has been reported [46]. Interestingly, the exact mechanism of the interface between membrane components and AgNPs is still unclear. Ag-generated free radicals resulting from the AgNPs surface are thought to have antimicrobial activity [58]. Lara and colleagues [59] proposed alternate bactericidal potential of the AgNPs based on protein inhibition as well as nucleic acid and cell wall synthesis [44]. It has been suggested by proteomic data that short term exposure of *E. coli* cells to AgNPs appeared in an aggregation of envelope protein precursors, indicating dissipation of proton motive force [59]. Thus, AgNPs have demonstrated the potential to diminish the outer membrane, reduce intracellular ATP and disrupt the plasma membrane potential. The mechanism of action of AgNPs and Ag+ ions appear to be similar at nanomolar and micromolar levels, respectively [60]. Studies on *E. coli* have also proposed that AgNPs possess antibacterial properties [61].

THE BROAD SPECTRUM OF ANTIMICROBIAL ACTIVITY OF AgNPs

Many researchers have proved the bactericidal efficacy of AgNPs (Table **1**), hence prompting the use of term "new generation of antimicrobials" [47]. Bactericidal potential of Ag^+ against *S. aureus* and *E. coli* has been reported by Feng *et al*. [62]. By studying matrix-assisted laser desorption ionization/time-of-flight mass spectrometry and two-dimensional electrophoresis and also employing energy filtering transmission electron microscopy, *E. coli* has been used as a model organism to study the bactericidal potential of Ag^+. Silver ion penetrates into the cells and influences the protein of ribosomal subunit and few enzymes vital for the cell [48].

De'Souza *et al*. reported antimicrobial potential of 19 antibiotics in a mixture with Ag-water dispersion solution (15 nm diameter AgNPs clusters containing Ag^+ produced by an electro colloidal Ag process) [63]. It was observed that the MDR *S. aureus, E. coli, Shigella flexneri, Bacillus subtilis* and *S. typhi* were more susceptible to the antimicrobial effects of clindamycin and amoxicillin. Surprisingly, the combination of Ag-water dispersion and clindamycin or amoxicillin had a preservative effect on *S. aureus* 6538P strain, *S. flexneri*, *B. subtilis* and *S. typhi*, while the mixture of Ag-water dispersion and amoxicillin exhibited combative effect with MRSA strain.

Duran *et al*. [64] synthesized AgNPs by fungus *Fusarium oxysporum* and reported that cotton fabrics saturated with AgNPs were adequate and firm bactericidals. The use of polyvinyl alcohol nano fibres impregnated with AgNPs in wound dressings had been tested and recommended as they were found to have antibacterial activity against *S. aureus* and *E. coli* [65]. Shahverdi *et al*. [45] reported an increased antibacterial potential of antibiotics with AgNPs. Ingle *et al*. [66] reported that Mycogenic AgNPs synthesized using *F. acuminatum* have 1.5-to-2 times stronger bactericidal potential than pure Ag^+ NPs in descending order against four human pathogenic microbes, viz. *S. aureus*, *S. epidermidis*, *S. typhi* and *E. coli*. AgNPs saturated with cellulose have antimicrobial potential against *S. aureus* and *E. coli* as found by Maneerung *et al*. [67]. Furthermore, Birla *et al*. [68] reported bactericidal

Table 1. Activity of Ag^+ ions and AgNPs against different bacterial pathogens.

S. No.	Forms of Ag	Bacterial pathogens embattled	Citations
1	AgNPs	*E. coli, S. aureus* and *P. aeruginosa*	Birla *et al*. (2009) [68], Bonde *et al*. (2011) [71]
2	AgNPs	*S. aureus, E. coli, K. pneumonia*	Gade *et al*. (2010) [70], Namasivayam *et al*. (2011) [75]
3	AgNPs	*E. coli, S. typhi, S. Epidermidis*, *S. aureus*	Ingle *et al*. (2008) [66]
4	Ag^+	*S. aureus, E.coli*	Feng *et al*. (2000) [62], Yamanaka *et al*. (2005) [48]
5	AgNPs coated medical device	*S. aureus* and *S. mutans*	Ki-Young (2011) [74]
6	AgNPs on cotton Fabrics	*S. aureus*	Duran *et al*. (2007) [64]
7	Nanofibers impregnated AgNPs	*E. coli* and *S. aureus*	Jun *et al*. (2007) [65]
8	AgNPs saturated on the wound dressing	*E. coli* and *S. aureus*	Maneerung *et al*. (2008) [67]
9	AgNPs	*E. coli* in liquid and solid medium	Baker *et al*. (2005) [43]
10	AgNPs	*E. coli*	Sondi and Salopek-Sondi (2004) [46]
11	AgNPs	*P. glomerata, P. herbarum, C. albicans*, *F. semitectum, Trichoderma sp.*	Gajbhiye *et al*. (2009) [69]
12	AgNPs	*S. aureus, E. coli*	Shahverdi *et al*. (2007) [45]
13	AgNPs	*E. coli, Vibrio cholerae, P. aeruginosa, S. typhus*	Morones *et al*. (2005) [18]

efficacy of AgNPs synthesized from *Phoma glomerata* and demonstrated that the NP biosynthesis is eco-friendly and NPs could solve the problem of ever rising resistance against antimicrobial agents.

Gajbhiye *et al*. [69] reported the extracellular biosynthesis of AgNPs by *Alternaria alternata* and studied the fungicidal activity of NPs alone as well as in combination with fluconazole and found that antifungal activity of fluconazole in formulation with AgNPs against *Phoma herbarum*, *Fusarium semitectum*, *P. glomerata*, *Candida albicans* and *Trichoderma sp*. was higher than when used alone. Similar activity was reported by Gade *et al*. [70] when it was observed that antibiotics used in combination with AgNPs showed remarkable antibacterial activity.

AgNPs phytosynthesis from the leaf extract of *Murraya koenigii*, an Indian curry leaf tree, was studied by Bonde *et al*. [71]. The bactericidal activity of synthesized NPs singly as well as in mixture with various antimicrobial agents against the pathogenic bacteria was studied and it was reported that activities of standard antibiotics improved considerably in the presence of AgNPs. Recently, Stefan *et al*. [72] tested antibacterial activity of AgNPs prepared from electrochemical synthesis in polyamide hydroxyurethane media against *E. coli* and *S. aureus* and observed that AgNPs at concentrations $5\mu gml^{-1}$ were strongly bactericidal against *S. aureus*.

Knetsch and colleagues [73] observed that medical devices coated with AgNPs inhibit bacterial adhesion and subsequent biofilm development. Hence, NPs can be deposited on the surface of a device from where Ag may gradually discharge and have bactericidal effects. Ki-Young [74] found that tissue conditioners having 0.1% and 0.5% of AgNPs showed bactericidal and fungicidal effects against *S. aureus*, *S. mutans* and *C. albicans*, respectively, while those above 1.0% of AgNPs had no viable cells. Namasivayam *et al*. [75] reported significant antibacterial activities of AgNPs (synthesized by *Candida glabrata* and *F. oxysporum)* against drug resistant pathogens like *E. coli*, *K. pneumoniae*, *Enterococcus faecalis*, *B. subtilis, S. aureus* and *P. aeruginosa*.

MECHANISM OF ACTION OF DIFFERENT ANTIBIOTIC GROUPS AND RESISTANCE STRATEGIES USED BY BACTERIA

An ideal antimicrobial agent should have selective toxicity without being detrimental to the host. Targets include biosynthetic functions and/or anatomic structures present exclusively in microorganisms rather than the host cell. There are several different mechanisms by which pathogens develop resistance including elimination of antibiotics through membrane efflux pumps, changes in cell wall permeability, drug action site modification, inactivation of drug, etc. [76, 77]. Aminoglycosides interfere with protein synthesis by binding to 30S subunit of the ribosome. Bacteria harboring transposon and plasmid encoded modifying enzymes can also inactivate the antibiotics [78]. Mechanism of action of different antibiotic groups and resistance strategies used by bacteria are described in Table **2**.

BACTERICIDAL EFFECT OF AgNPs AGAINST MDR BACTERIA

AgNPs show antimicrobial activity against MDR organisms and are used as valuable antimicrobial agents. AgNPs were evaluated for their bactericidal potential against different bacteria (including MDR microbes like MRSA) and it was found that colloidal AgNPs were potent bactericidals [83]. AgNPs can be utilized as broad spectrum antibacterial agents against Gram-positive and Gram-negative bacteria as well as antibiotic resistant bacteria like

Table 2. Mechanism of action of different antibiotic groups and resistance strategies used by bacteria.

Antibiotic Class	Mechanism of action	Resistance strategy used by bacteria	Citations
Aminoglycoside antibiotics	Inhibit synthesis of protein by binding to 30S subunit of ribosome	Plasmid and transposon modifying enzymes inactivate the encoded antibiotics	Kotra *et al*. (2000) [78]
Beta-lactam antibiotics	Inhibit peptidoglycan layer synthesis of cell wall	Production of Beta lactamase to impair the Beta-lactams	Poole (2004) [79]
Sulfonamide antibiotics	Interfere with folic acid metabolism by competitively suppressing bacterial alteration of para-aminobenzoic acid into dihydrofolate	O Owing to acquisition of plasmid that en code a drug-resistant Dihydropteroate	Chopra (2007) [80]
Phenicol antibiotics	Prevent transpeptidation of peptide chain elongation by binding reversibly to the peptidyltransferase component of the 50S ribosomal subunit	A Acquisition of plasmids encoding c chloramphenicol acetyltransferases w which enzymatically inactivate the dr drug	Falagas *et al*. (2008) [81]
Tetracycline antibiotics	Blocks the access of aminoacyl t-RNA to the RNA-ribosome complex by reversibly binding to the 30S ribosomal subunit to prevent polypeptide synthesis	Outer membrane permeability affected due to chromosomal mutations	Chopra (2007) [80] Falagas *et al*. (2008) [81]
Quinolones fluoroquinolones	Interfere with DNA replication by targeting DNA gyrase	Gene mutation of target and removal by efflux pumps	Hooper (2000) [82]

MRSA and vancomycin resistant *S. aureus* and *E. faecium* (forming biofilms) [84]. AgNPs prevent formation of biofilms and, thus, prevent the bacteria from developing resistance.

By performing minimal inhibitory concentration (MIC) and minimal bactericidal concentration tests in LB broth using NPs, bactericidal activity of AgNPs against MRSA and non-MRSA has been reported [85]. Similarly, bactericidal effects of AgNPs against *S. mutans* (causal agent for dental caries) have also been reported [86].

Nanda and Saravanan [87] reported AgNPs (synthesized by aqueous Ag^+ reduction with *S. aureus*) were most potent against MRSA followed by MRSE and *S. pyogenes*, but only moderately potent against *S. typhi* and *K. pneumoniae*. Similarly, maximum activity was demonstrated against drug resistant MRSA, followed by drug resistant MRSE. AgNPs are shown to have both bactericidal and bacteriostatic effects against *S. aureus*, methicillin sensitive *S. aureus* and MRSA [88]. MIC (broth micro dilution method) of colloidal AgNPs synthesized by sol-gel method was found to be 2-4 μg ml^{-1} against pathogens like *C. albicans*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae* and *S. typhimurium* [89]. The bactericidal potential of AgNPs against MDR microbes has been illustrated in Table **3**.

APPLICATION OF GOLD NANOPARTICLES (AuNPs) AND THEIR ANTIMICROBIAL ACTIVITY: A GENERAL VIEW

The remedial use of gold (Au) dates back to 2500 BC where Chinese used it in medicines. Red colloidal Au by name of Swarna Bhasma ("Swarna" meaning gold, "Bhasma" meaning ash) is used in the Indian Ayurvedic Medicine [90, 91]. Historically, use of Au has been recorded as nervine agent (for neural conditions) as well as therapeutic agent in epilepsy (19th century) and syphilis (16th century). Au based remedy for tuberculosis was popularized in the 1920s after its bacteriostatic properties were discovered by Robert Koch [92]. Even today, the use of Au compounds in the cure of rheumatic diseases along with juvenile arthritis, psoriasis and planindromic rheutamitism [93] is widespread. Due to their biocompatibility, Au particles are useful [94, 95]. AuNPs, though being biologically inert, can be engineered to acquire photothermal/chemical functionality as it is evident by the effectiveness of infrared (NIR) irradiated Au-based nanomaterials, Au nanocages, Au nanospheres and Au nanorods with characteristic NIR absorption against cancer cells and pathogens. In combination with photosensitizers (like hydrophilic toluidine blue O), AuNPs (Au nanorods serving both as photodynamic and photothermal agents) can be used in photodynamic antimicrobial chemotherapy against microbes like MRSA and also in NIR photothermal radiation. Photodynamic antimicrobial chemotherapy (PACT) and hyperthermia in combination have enhanced the antimicrobial effect of AuNPs [96-99]. Light-absorbing AuNPs attached with definite antibodies have also been exploited to photothermally kill *S. aureus* using laser [100]. AuNPs have been emphasized in recent studies as photothermal agents for killing pathogens hyperthermically [101-103]. The efficiency of the antibacterial activity of AuNPs can be improved by adding antimicrobial agents [104]. Various studies have shown that antibiotics coated with AuNPs have enhanced antimicrobial activity [105-107]. For example, Cefaclor coated with AuNPs is effective against Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*). It renders bacterial cell wall porous by inhibiting synthesis of peptidoglycan layer while AuNPs cause holes in the cell wall, eventually leading to leakage of cell contents and cell death. Another possible effect is inhibition of uncoiling and transcription of bacterial DNA by AuNPs [108]. Implants, fabrics for cure of wounds and glass surfaces coated with AuNPs can be used to sustain hygienic conditions in the hospitals and at homes [109].

AuNPs have also been analyzed for their anti-HIV activity [110]. Infection of T-cells by HIV is inhibited *in vitro* by AuNPs (nanomolar concentrations) coated with multiple copies of an am-

Table 3. Activity of AgNPs against MDR bacteria.

S. No.	MDR bacterial pathogens targeted	Citation
1	MRSA	Panacek *et al*. (2006) [83]
2	MRSA and non-MRSA	Ayala-Nunez *et al*.(2009) [85]
3	MRSA, MRSE and *S. pyogenes*	Espinosa-Cristobal *et al*. (2009) [86]
4	MRSA and MRSE	Nanda and Saravanan (2009) [87]
5	Erythromycin-resistant *S. pyogenes*, ampicillin-resistant *E. coli*, MDR *P. aeruginosa*	Nanda and Saravanan (2009) [87]
6	*S. aureus*, methicillin sensitive *S. aureus* (MSSA), and MRSA	Humberto *et al*. (2010) [3]
7	*E. coli*, *S. aureus*, *C.* albicans, *B. subtilis*, *S. typhimurium*, *P. aeruginosa* and *K. pneumoniae*	Ansari *et al*. (2011) [88]
8	*S. epidermidis*, *S. typhi*, *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *E. coli*, *K. Pneumoniae*	Lkhagvajav *et al*. (2011) [89]

phiphilic sulfate-ended ligand that binds envelope glycoprotein gp120, thus making multivalent AuNPs coated with sulfated ligands a novel alternative to the recognized anti-HIV systems. NPs can bind gp120 with high affinity depending on the concentration of ligand [111]. The possible interactions between the sulfated ligands and other active molecules on same nanoplatform qualify AuNPs as an interesting scaffold which can give rise to the advancement of innovative multifunctional anti-HIV systems [112]. The exercise of AuNPs with diverse anionic groups (depending upon charge density and functional groups) to inhibit influenza by simply blocking attachment of virus to the cell surface has been reported [113]. The small size of the AuNPs facilitates entry into the cell through the endosome vesicles, hence allowing them to possibly impede with the fusion step as well [114]. Various studies have established that there is little or no cytotoxicity associated with AuNPs and if there is any toxicity present then it is dependent on the surface charge, shape and size of the particle [115]. The NPs composed of an Au core with thiol group-bound mercaptoethanesulfonate (MES) molecules to its surface has been shown to inhibit multiple strains of influenza including even those which undergo random genetic mutations like recent pandemic swine influenza A (H1N1) strain.

Various modifications like non-covalent interaction of AuNPs (like citrate-capped) with antibiotics [116, 117] and/or reduction of Au chloride in the presence of antimicrobial agents [107] enhance the bactericidal activities of NPs. Free amino group antibiotics have stronger compatibility to Au surface leading to aggregation as is evident by change in color from red to blue/purple. There are contradicting reports about the effectiveness of NPs aggregates in terms of bactericidal activity, some suggesting enhanced potency whereas others report little or no measurable increase in potency [104]. Interestingly, for NP conjugates to be useful, it has been suggested that their functionalization should be such so as to prevent aggregation which would increase surface coverage on the NPs surface. To support this contention, it has been suggested that there should be formation of physical cross-linkages and uncontrolled agglomeration of particles while mixing AuNPs with molecules having multiple amino groups. Although it may not be the only factor influencing the stability, by using a system with only a single amino group, the degree of aggregation could still be decreased [108] using Cefaclor., As against the previously suggested reports that AuNPs are significant only as scaffolds to compactly bind antimicrobial agents, Rai *et al*. argued that these also strengthen the damage of cell membrane initiated by the antibiotics, increase the diffusion into bacteria and also inhibit the bacterial DNA.

METAL OXIDE NPs AND THEIR ANTIMICROBIAL ACTIVITY

Inherent antimicrobial properties of metal oxide NPs have attracted the attention of researchers. Maximum antibacterial effects of smallest (8 nm) magnesium oxide NPs have been reported against *S. aureus* and *E. coli* [118]. Similarly, antimicrobial properties of iron oxide NPs formulated into nanometer rather than micron have also been reported in dose-dependent manner [119]. Interestingly, the lesser the particle size, the greater the antibacterial effects as reported for Zinc oxide (ZnO). Direct connection between antibacterial activity, particle size and surface area of ZnO is reported by nitrogen gas (N_2) isotherms and the Brunauer-Emmett-Teller equation in the relative pressure range (P/Po) of 0.05-0.30, representing that colloidal suspension with the maximum surface area (90.4 m^2/g) can inhibit 95% of *E. faecalis*, MRSA, *S. epidermidis* (a high-biofilm-producing strain), apart from other pathogen growths [120]. However, it was also noted in this study that the suspension of ZnO was much less efficient against *S. typhimurium*, suppressing only half of the growth. This is a matter of concern because this strain is responsible for various infections and outbreaks including the pea nut related incidence reported in 2009 [121]. Thus, future studies should focus on the possible mechanism of ZnO NPs resistance being developed by *S. typhimurium.*

Antibacterial effects of NPs could be attributed to reactive oxygen species (ROS) production [122] by the release of metal ion (related to surface area) and the effect of metal oxide NPs or through their contact with ultraviolet (UV) light [123] as is evident from various studies. One such example is Fenton reaction which converts hydrogen peroxide (H_2O_2) to the additional reactive hydroxyl radical [124] which has multiple deleterious effects [125]. Higher surface areas of nanometer size particles in comparison with micron particles accelerate the processes especially when NPs penetrate the pathogen.

Lipovsky and colleagues found that ZnO NPs tend to generate more singlet oxygen and hydroxyl radicals in water suspensions. Conversely, when the suspension was irradiated with visible light (400-500 nm), more oxy radicals were generated, representing the capability of non destructive visible light to stimulate ROS production in ZnO [126]. Possible mechanism of H_2O_2 production by ZnO was not discounted as reported in earlier studies and re-

searches [122, 127, 128] which is the major limitation of the above study.

Nanomaterials generated ROS can affect the integrity of cell membrane and metabolic activity. The exact mechanism of interaction between functionalized nanomaterials and microorganisms/biosystems is still in rudimentary stages and advance research is required [129], which will open new frontiers in the field of biomedicine.

CONCLUSION

Resistance to drug/antibiotics has resulted in the recurrence of parasites and MDR microbes. Infections produced by such strains involve multi-regime therapy which may have less efficacy, more side effects and higher costs. Recent advances and multidisciplinary researches in the field of nanomedicine provide us an insight into the mechanisms of drug resistance and help the researchers to devise newer ways to solve the problem at hand. Specifically, NPs of Au, Ag and metal oxides have wide applications in diagnostic sensors, as antimicrobials and as agents in drug and gene delivery. Much need to be done to unravel the structure and functions of biosystems at the nanoscale which in turn would improve the quality of research and prove beneficial to life sciences particularly health care system. Advantage of nanomaterials lies in the fact that these can be used both *in vivo* and *in vitro*. Possible mechanisms by which NPs manifest antibacterial activity is by attaching and piercing the cell wall of pathogens and through modulating signaling pathways possibly by causing dephosphorylation of putative key peptide substrates on tyrosine residues. This knowledge can be effectively applied for the cure and avoidance of drug-resistant microbes.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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LIST OF ABBREVIATIONS

MDR	=	Multidrug resistant
NPs	=	Nanoparticles
MDRB	=	Multiple drug resistant bacteria
ROS	=	Reactive oxygen species
AgNPs	=	Silver nanoparticles
MRSA	=	Methicillin-resistant *Staphylococcus aureus*
Au	=	Gold
AuNPs	=	Gold nanoparticles
Ag	=	Silver
MRSE	=	Methicillin-resistant *Staphylococcus epidermidis*
ZnO	=	Zinc oxide

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