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REVIEW ARTICLE

Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria

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Summary

In the present scenario, pharmaceutical and biomedical sectors are facing the challenges of continuous increase in the multidrug-resistant (MDR) human pathogenic microbes. Re-emergence of MDR microbes is facilitated by drug and/or antibiotic resistance, which is acquired way of microbes for their survival and multiplication in uncomfortable environments. MDR bacterial infections lead to significant increase in mortality, morbidity and cost of prolonged treatments. Therefore, development, modification or searching the antimicrobial compounds having bactericidal potential against MDR bacteria is a priority area of research. Silver in the form of various compounds and bhasmas have been used in Ayurveda to treat several bacterial infections since time immemorial. As several pathogenic bacteria are developing antibiotic resistance, silver nanoparticles are the new hope to treat them. This review discusses the bactericidal potential of silver nanoparticles against the MDR bacteria. This multiactional nanoweapon can be used for the treatment and prevention of drug-resistant microbes.

Introduction

Resistance in human pathogens is a big challenge in fields like pharmaceutical and biomedicine. Antibiotic resistance profiles lead to fear about the emergence and re-emergence of multidrug-resistant (MDR) pathogens and parasites (Tenover 2006). Once an individual is infected with MDR bacteria, it is not possible to cure easily and he/she has to spend more time in the hospital and requires a multiple treatment of broad-spectrum antibiotics, which are less effective, more toxic and more expensive (Webb *et al.* 2005). Therefore, development of or modification in antimicrobial compounds to improve bactericidal potential is a priority area of research in this modern era (Humberto *et al.* 2010). Nanotechnology provides a good platform to modify and develop the important properties of metal in the form of nanoparticles having promising applications in diagnostics, biomarkers, cell labelling, contrast agents for biological imaging, antimicrobial agents, drug delivery systems and nanodrugs for treatment of various diseases (Marcato and Duran, 2008; Singh and Singh 2011). Hence, researchers are shifting towards nanoparticles in general and silver nanoparticles in particular to solve the problem of emergence of MDR bacteria (Gemmell *et al.* 2006).

Silver has a strong antimicrobial potential, which has been used since the ancient times. But with the advent of antibiotics progress, the medical applications of silver as antimicrobial were declined (Castellano *et al.* 2007; Chen and Schluesener 2008). Antimicrobial effects of silver can be increased by manipulating their size at nanolevel. Because of their change in physiochemical properties, silver nanoparticles have emerged as antimicrobial agents owing to their high surface-area-to-volume ratio and the unique chemical and physical properties (Kim *et al.* 2007). Silver nanoparticles having size in the range of 10–100 nm showed strong bactericidal potential against both Gram-positive and Gram-negative bacteria (Morones *et al.* 2005). The bactericidal activity of silver nanoparticles against the pathogenic, MDR as well as multidrug-susceptible strains of bacteria was studied by many scientists, and it was proved that the silver nanoparticles are the powerful weapons against the MDR bacteria such as *Pseudomonas aeruginosa*, ampicillin-resistant *Escherichia*

coli, erythromycin-resistant *Streptococcus pyogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *vancomycin*-resistant *Staphylococcus aureus* (VRSA).

Silver-based antimicrobials: prehistorical, historical and current status

Silver is a basic, rare and naturally occurring element, which is slightly harder than gold, very ductile and malleable, having the highest electrical and thermal conductivity with minimum contact resistance within all metals (Susan *et al.* 2009). Since the primeval times, silver has been used for making utensils, jewelery, dental alloy, photography, monetary currency, explosives, etc. (Chen and Schluesener 2008). Before the beginning of antibiotics therapy, silver was used for its antiseptic activity, specifically for the treatment for open wounds and burns (Moyer *et al.* 1965). Ancient people were much aware of the bactericidal properties of silver (Susan *et al.* 2009). The silver ions are highly reactive, and they bind to proteins followed by structural changes in the bacterial cell wall and nuclear membrane, which leads to cell distortion and death. Silver ions have capacity to inhibit the bacterial replication, by binding and denaturing bacterial DNA (Landsdown 2002; Castellano *et al.* 2007). Silver ions react with thiol group of proteins, followed by DNA condensation resulting in the cell death (Feng *et al.* 2000). Various types of silver compounds that are used as antimicrobials from ancient times include silver nitrate, silver sulfadiazine, silver zeolite, silver powder, silver oxide, silver chloride and silver cadmium powder.

Silver nitrate ($AgNO_3$)

Silver nitrate is the solid compound of silver and known by different names in different languages as 'Lunar caustic' (English), 'Lapis infernalis' (Latin) and 'Pierre infernale' (French). Silver nitrate was used for the treatment for venereal diseases, fistulae from salivary glands and bone and perianal abscesses in 1700s (Landsdown 2002). In the 19th century, silver nitrate was used to treat the burns, and it was believed that silver nitrate allows epithelization and promotes crust formation on the surface of wounds (Castellano *et al.* 2007). In 1881, silver nitrate eye drops was used by Carl S. F. Crede to cure ophthalmia neonatorum; later, B. Crede designed silver-impregnated dressings for skin grafting (Landsdown 2002).

In 1884, aqueous silver nitrate drops were used to prevent the transmission of *Neisseria gonorrhoeae* from infected mothers to children during childbirth (Silvestry-Rodriguez *et al.* 2007). Silver nitrate in the form of 0·5% solution was used for the treatment for burns, stating that this solution have strong bactericidal potential against *Staph. aureus*, *Ps. aeruginosa* and *E. coli* and does not interfere with epidermal proliferation (Bellinger and Conway 1970).

Silver sulfadiazine (AgSD)

It is the combination of silver with sulfadiazine, generally used as a 1% water-soluble cream, which is a broad-spectrum bactericidal. It is used especially for the treatment of burn wounds. Its mode of action is membrane-damaging by binding to cell components including DNA (Atiyeh *et al.* 2007) and transcription inhibition by binding to the base pairs in DNA helix (Maple *et al.* 1992; Mcdonnell and Russell 1999). Silver sulfadiazine cream was prepared for the treatment of burns, which also demonstrated strong antibacterial potential against *E. coli, Staph. aureus, Klebsiella* sp. and *Pseudomonas* sp.

Silver zeolite

Silver zeolite is a complex made up of alkaline earth metal and crystal aluminosilicate, partially replaced by silver ions using ion exchange phenomenon. In Japan, ceramics have silver zeolite coat, having bactericidal properties that protect the products, and are used in food preservation, disinfection of medical products and decontamination of materials (Kawahara *et al.* 2000; Matsumura *et al.* 2003). Matsumura *et al.* (2003) suggested two possible modes of action of silver zeolite: (i) when bacterial cells come in contact with silver zeolite, cells take in silver ions, which ultimately damage the bacterial cell; and (ii) generation of reactive oxygen species, by which inhibition of respiratory enzyme takes place with the help of silver ions leads to bacterial cell damaging.

Nanoparticles

The nanosize of material results in specific physicochemical characteristics different than those of the bulk materials or larger particles. This effect is mainly credited to high surface-area-to-volume ratio, which results in increased reactivity; hence, the nanoscale materials are more advantageous than their bulk materials. The metallic nanoparticles such as copper, titanium, magnesium, zinc, gold and alginate have a strong bactericidal potential owing to their large surface-area-to-volume ratio (Gu *et al.* 2003; Ahmad *et al.* 2005). Among all, silver nanoparticles have proved to be the most effective antimicrobial agent against bacteria, viruses and other eukaryotic micro-organisms (Gong *et al.* 2007).

Silver nanoparticles

Silver nanoparticles of size smaller than 100 nm contain about 10 000–15 000 silver atoms (Oberdorster *et al.* 2005; Warheit *et al.* 2007). They are prepared by engineering the metallic silver into ultrafine particles by numerous physical methods, which include spark discharging, electrochemical reduction, solution irradiation and cryochemical synthesis (Chen and Schluesener 2008). Similarly, chemical and biological methods can be used for the synthesis of silver nanoparticles. The silver nanoparticles exhibit important physicochemical properties, such as pH-dependent partitioning to solid and dissolved particulate matter, and biological activities, when compared with the regular metal (Lok *et al.* 2006; Pal *et al.* 2007). Since the ancient time, silver nanoparticles have emerged as antimicrobial agents owing to their high surface-area-to-volume ratio and the unique chemical and physical properties (Morones *et al.* 2005; Kim *et al.* 2007).

Silver nanoparticles have important biological properties as follows: they are effective bactericidal agents against broad spectrum of bacteria, including antibiotic-resistant strains (Percival *et al.* 2007), fast-acting fungicide against common fungi including *Aspergillus*, *Candida* and *Saccharomyces*. Silver nanoparticles of 5–20 nm diameter can inhibit HIV-1 virus replication. (Sun *et al.* 2005; Humberto *et al.* 2010). These can not only alter the expression proteinases, which are important in inflammatory and repair processes, but also suppress tumour necrosis factor (TNF), interleukin (IL)-12 and IL-1b and induce apoptosis of inflammatory cells (Bhol *et al.* 2004). Moreover, silver nanoparticles are also responsible for cytokine modulation in wound healing (Tian *et al.* 2007) and inhibition of the biofilm formation.

Silver nanoparticles: the broad spectrum of antimicrobials

Bactericidal efficacy of silver nanoparticles was investigated by many researchers and their effective potential against broad range of microbes was proved (Table 1), including antibiotic-resistant bacteria. Silver nanoparticles are also termed as new-generation of antimicrobials (Rai *et al.* 2009). The group of researchers actively proved the bactericidal potential of silver nanoparticles. Feng *et al.* (2000) reported the bactericidal potential of silver ions against *Staph. aureus* and *E. coli*. Sondi and Salopek-Sondi (2007) also demonstrated bactericidal activity of silver nanoparticles against *E. coli* as a model for Gram-negative bacteria. They observed formation of aggregates composed of silver nanoparticles and dead bacterial cells in the SEM analysis by performing the TEM analysis and EDAX study, concluded that silver nanoparticles interact with the building elements of the bacterial cell membrane, leading to cell damage.

Yamanaka *et al.* (2005) investigated the bactericidal potential of silver ions using *E. coli* as a model organism with the help of energy-filtering TEM and by studying two-dimensional electrophoresis and matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI-TOF MS). From their study, it was confirmed that the silver ions penetrate into the bacterial cells and affect the ribosomal subunit protein and some enzymes important for the bacterial cell (Yamanaka *et al.* 2005). Baker *et al.* (2005) synthesized nanoparticles by inert gas condensation and co-condensation techniques and studied the antibacterial potential of nanoparticles against *E. coli* in liquid and solid media, concluding that the nanoparticles were cytotoxic to *E. coli*. In 2005, Morones *et al.* performed a study on bactericidal effect of silver nanoparticles of size 1–100 nm on Gram-negative bacteria *E. coli*. They analysed the interaction of silver nanoparticles with bacteria by growing the bacterial cells up to mid-log phase, measuring OD at 595 nm, studied the effect of different concentrations of silver on bacterial growth and observed that concentration more than 75 μg ml^{-1} was lethal for bacteria (Kim *et al.* 2007).

De'Souza studied the antimicrobial activity of 19 antibiotics in combination with the silver–water dispersion solution (15-nm-diameter silver nanoparticle clusters containing silver ions produced by an electrocolloidal silver process). They found that the MDR *E. coli*, *Staph. aureus*, *Salmonella typhi, Shigella flexneri* and *Bacillus subtilis* are susceptible to amoxicillin and clindamycin. Interestingly, the combination of silver–water dispersion and amoxicillin or clindamycin showed an additive effect on *Staph. aureus* 6538 P strain, *Salm. typhi, Sh. flexneri* and *B. subtilis*, while combination of silver–water dispersion and amoxicillin showed antagonistic effect with methicillin-resistant *Staph. aureus* strain (MRSA) (De Souza *et al.* 2006).

Duran *et al.* (2007) synthesized silver nanoparticles using fungus *Fusarium oxysporum*, studied bactericidal activities of silver nanoparticles by incorporating them on textile fabric and confirmed them with the help of spectroscopic techniques scanning electron microscopy (SEM) and energy-dispersive spectroscopy. They reported that the cotton fabrics impregnated with silver nanoparticles possess efficient and strong bactericidal potential. Polyvinyl alcohol nanofibres impregnated with silver nanoparticles have strong and efficient antibacterial potential against *E. coli* and *Staph. aureus*, and its use in wound dressings was recommended and tested (Jun *et al.* 2007). Shahverdi *et al.* (2007) studied the effect of silver nanoparticles and antibiotics combination on the bacteria. They synthesized the silver nanoparticles using *Klebsiella*

Table 1 Activity of silver nanoparticles against broad spectrum of bacteria

S. No.	Different forms of silver	Target organisms	References
1.	Silver ions	*Staphylococcus aureus* and *Escherichia coli*	Feng *et al.* (2000)
2.	Silver nitrate	Periodontal pathogens	Spacciapoli *et al.* (2001)
3.	Silver zeolite	*E. coli*	Matsumura *et al.* (2003)
4.	Silver nanoparticles	*E. coli*	Sondi and Salopek (2007) Pal *et al.* (2007)
5.	Silver ions	RNA viruses	Butkus *et al.* (2004)
6.	Silver nanoparticles	*E. coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Salmonella typhus*	Morones *et al.* (2005)
7.	Silver nanoparticles	*E. coli* in liquid and solid medium	Baker *et al.* (2005)
8.	Silver ions	*E. coli*	Yamanaka *et al.* (2005)
9.	Silver nanoparticles	*Staph. aureus* and *E. coli*	Shahverdi *et al.* (2007)
10.	Super paramagnetic silver nanoparticles, bifunctional Fe_3O_4, @Ag nanoparticles	*E. coli, Bacillus subtilis* and *Staphylococcus epidermidis*	Gong *et al.* (2007)
11.	Nanofibres impregnated silver nanoparticles	*E. coli* and *Staph. aureus*	Jun *et al.* (2007)
12.	Silver nanoparticles on cotton Fabrics	*Staph. aureus*	Duran *et al.* (2007)
13.	Silver nanoparticles impregnated on the wound dressing	*E. coli* and *Staph. aureus*	Maneerung *et al.* (2008)
14.	Silver nanoparticles	*E. coli*, *Salmonella typhi*, *Staphylococcus epidermidis Staph. aureus*	Ingle *et al.* (2008)
15.	Silver nanoparticles	*Phoma glomerata*, *Phoma herbarum, Fusarium semitectum, Trichoderma* sp. and *Candida albicans*	Gajbhiye *et al.* (2009)
16.	Silver nanoparticles	*E. coli*, *Staph. aureus* and *Ps. aeruginosa*	Birla *et al.* (2009)
17.	Silver nanoparticles	*E. coli* and *Staph. aureus*	Gade *et al.* (2010)
18.	Silver nanoparticles	*E. coli* and *Ps. aeruginosa*	Geethalakshmi and Sarada (2010)
19.	Silver nanoparticles	*E. coli*, *Staph. aureus* and *Ps. aeruginosa*	Bonde *et al.* (2011)
20.	Silver nanoparticles	*Ps. aeruginosa*, *Staph. aureus*, pathogenic fungi *Aspergillus flavus* and *Aspergillus niger*	Govindaraju *et al.* (2010)
21.	Silver nanoparticles	*Staph. aureus*, *E. coli, Klebsiella pneumoniae, B. subtilis, Enterococcus faecalis, Ps. aeruginosa*	Namasivayam *et al.* (2011)
22.	Silver nanoparticles coated medical devices	*Staph. aureus* and *Streptococcus mutans*	Ki-Young (2011)
23.	Bacterial cellulose-silver nanoparticles composite	*E. coli* and *Staph. aureus*	Hernane *et al.* (2011)

pneumoniae and evaluated their antimicrobial activity alone and in combination with the antibiotics such as penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin against *Staph. aureus* and *E. coli*. They observed a significant increase in antibacterial activity of antibiotics in the presence of silver nanoparticles, and there was highest synergistic activity of nanoparticles with erythromycin against *Staph. aureus* (Shahverdi *et al.* 2007).

Synthesis of the silver nanoparticles by *Fusarium acuminatum* and study of the bactericidal efficiency of silver nanoparticles against four human pathogenic bacteria, viz. *E. coli*, *Salm. typhi*, *Staphylococcus epidermidis* and *Staph. aureus*, were carried out by Ingle *et al.* (2008). Mycogenic silver nanoparticles have bactericidal potential against each of the above bacterium, and the effect was 1·5–2 times stronger than that of pure silver ions. They found the maximum antibacterial activity of silver nanoparticles against *Staph. aureus*, followed by *Staph. epidermidis* and *Salm. typhi* and the minimum by *E. coli*. Silver nanoparticles impregnated with bacterial cellulose providing their antimicrobial activity against *E. coli* and *Staph. aureus* were reported by Maneerung *et al.* (2008). Extracellular synthesis of silver nanoparticles from *Phoma glomerata* and study of its bactericidal efficacy against *E. coli*, *Staph. aureus* and *Ps. aeruginosa* were carried out by Birla *et al.* (2009). The authors studied the antibacterial activity of silver nanoparticles in combination with antibiotics against *E. coli*, *Staph. aureus* and *Ps. aeruginosa*. The authors found that silver nanoparticles in combination with antibiotics showed strong antibacterial activity against the bacteria exhibiting resistance to various antibiotics and concluded that the biosynthesis route of nanoparticle synthesis is eco-friendly and these nanoparticles are the best solution for the increasing bacterial resistance against antibiotics.

Gajbhiye *et al.* (2009) carried out extracellular biosynthesis of silver nanoparticles using *Alternaria alternata*.

The authors further studied the fungicidal activity of nanoparticles alone and in combination with commercially available antifungal agent fluconazole. They found that not only silver nanoparticles can inhibit the fungal growth, proving its antifungal properties, but also there was increase in the antifungal activity of fluconazole in combination with silver nanoparticles against the *P. glomerata*, *Phoma herbarum*, *Fusarium semitectum*, *Trichoderma* sp. and *Candida albicans*. Gade *et al.* (2010) synthesized silver nanoparticles from *Opuntia ficus-indica* and evaluated antibacterial activity of silver nanoparticles against *E. coli* and *Staph. aureus*. They found that the silver nanoparticles in combination with commercially available antibiotics demonstrated remarkable antibacterial activity.

Phytosynthesis of silver nanoparticles from the leaf extract of *Murraya koenigii*, an Indian curry leaf tree, was reported, and the bactericidal potential of synthesized nanoparticles singly and in combination with commercially available antibiotics gentamycin, ampicillin, tetracycline and streptomycin against the pathogenic bacteria, viz. *E. coli*, *Staph. aureus* and *Ps. aeruginosa* was studied (Bonde *et al.* 2012). They observed that silver nanoparticles in combination with gentamycin showed the maximum activity against *E. coli* with an increase in fold area 4·06. While tetracycline combination with nanoparticles showed maximum activity against *Staph. aureus* (2·16), they concluded that activity of standard antibiotics was significantly increased in the presence of silver nanoparticles and that can be used against antibiotic-resistant pathogens effectively. Recently, antimicrobial activity of the silver nanoparticles of 19–23 nm size prepared from electrochemical synthesis in polyamide-hydroxyurethane media was investigated (Stefan *et al.* 2011). The antibacterial activity was tested by disc diffusion method against *E. coli* and *Staph. aureus*. They found that silver nanoparticles of size 23 nm at concentrations 5 μg ml^{-1} were strong bactericidal against *Staph. aureus*.

Knetsch *et al.* (2011) reviewed that if the surface of medical devices has a coating of silver nanoparticles, then it was helpful to prevent bacterial adhesion and subsequent biofilm formation on the medical devices. These nanoparticles can directly be coated and/or deposited directly on the device surface; where silver is slowly released from the surface and kills the bacterial population near it. Ki-Young (2011) prepared a combination of silver nanoparticles with tissue conditioner and tested its antibacterial activity against *Staph. aureus*, *Streptococcus mutans* and *C. albicans*. It was found that only 0·1% of silver nanoparticles combination with tissue conditioner showed bactericidal effect against two bacterial strains, *Staph. aureus* and *Strep. mutans*. There were no viable cells above 1·0% of silver nanoparticles. Tissue conditioner with 0·5% silver nanoparticles had fungicidal potential when tested against *C. albicans*. There was no CFU observed above 2·0%. Namasivayam *et al.* (2011) reported the synthesis of silver nanoparticles mainly by using *Candida glabrata* and *F. oxysporum*. The authors evaluated these silver nanoparticles against drug-resistant pathogenic bacteria *Staph. aureus*, *E. coli, Kl. pneumoniae, B. subtilis, Enterococcus faecalis* and *Ps. aeruginosa*. They observed that the synthesized silver nanoparticles showed significant antibacterial activity against all the pathogenic bacteria (Table 1).

Silver nanoparticles against multidrug-resistant bacteria

Antibiotic resistance

The bacteria have powerful ability of developing resistance against antibiotics, which is the natural gift to them before the discovery and implementation of the penicillin in 1929 by Sir Alexander Fleming. However, in recent scenario, the bacterial resistance profile is continuously increasing and its control is the major challenge for scientists and researchers. World is facing a global problem of increase in the antibiotic resistance, owing to wide and often indiscriminate use of antibiotics and pesticides and related compounds in agriculture. Institute of Food Technology, England, defined the drug resistance as the 'temporary or permanent ability of a micro-organism and its progeny to remain viable and/or multiply under conditions that would destroy or inhibit other members of the strain'. Abraham and Chain (1940) reported that the drug resistance in bacteria was attributable to the presence of different kinds of enzyme in bacteria and also warned that the misuse of antibiotics could lead to the development of resistance and the propagation of mutant strains, resistant to antibiotics in the environments. Once the bacteria come into contact with antibiotic, but not killed by it, bacteria can develop resistance by adapting their cell structure or metabolism to destroy the antibiotic in future. Hence, bacterial exposure to antimicrobials are the opportunities for bacteria to acquire resistance. There are certain methods of acquiring resistance, which include genetic mutation, modification of present genetic material or gaining of new genetic material. Once resistance is acquired by bacteria, it can share, exchange and transfer vertically (to its progeny) or horizontally (to neighbouring bacteria) by transduction (bacteriophage-mediated transfer of DNA between two bacteria), transformation (DNA are taken up by bacteria from the external environment) or by conjugation (direct cell-to-cell contact to transfer DNA; Slonczewski and Foster, 2009).

Mechanisms of antimicrobial action and resistance

Selective toxicity is an important feature of an ideal antimicrobial drug in which the drug is harmful only to the parasite without being harmful to the host. Targets of ideal antimicrobial agents are anatomic structures and/or biosynthetic functions present uniquely in micro-organisms rather than the host cell. Development of resistance to antimicrobial agents in bacteria is mediated by several mechanisms, which mainly include changes in bacterial cell wall permeability, removal of antimicrobial agents through efflux pumps of membrane, drug action site modification, antimicrobial agent's inactivation, etc. (Cebrian *et al.* 2003; Biyela *et al.* 2004).

The mechanisms of action and resistance of major categories of antimicrobial agents are described in Table 2.

Silver nanoparticles' bactericidal effect against multidrug-resistant bacteria

Silver nanoparticles are used as effective antimicrobial agents. They have bactericidal potential against MDR organisms.

Panacek *et al.* (2006) synthesized silver nanoparticles by developing one-step protocol and evaluated their antimicrobial activity against Gram-positive and Gram-negative bacteria, including MDR strains such as MRSA. Colloidal silver nanoparticles were found to possess significant bactericidal potential against MRSA and Gram-positive and Gram-negative bacteria. According to Percival *et al.* (2007), silver nanoparticles can be used as effective broad-spectrum antibacterial agents for Gram-negative and Gram-positive bacteria, including antibiotic-resistant bacteria. Gram-negative bacteria include members of the genera *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella* and *Vibrio*. Gram-positive bacteria include *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus* and *Streptococcus*. Antibiotic-resistant bacteria include methicillin- and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA) and *Enterococcus faecium*, by preventing biofilm formation, which act as efficient barriers against antimicrobial agents and the host immune system to protect the bacterial colony. In a study of antibacterial activity of the silver nanoparticles against MRSA and non-MRSA, minimal inhibitory concentration (MIC) and minimal bactericidal concentration were evaluated in LB broth using nanoparticles of 100 nm (Ayala-Nunez *et al.* 2009). They observed the dose-dependent bactericidal activity of silver nanoparticles against MRSA and non-MRSA and

Table 2 Mechanisms of action and resistance of major categories of antimicrobial agents

Antimicrobial group with examples	Mode of action	Mechanism of resistance	References
1. Beta-lactams monobactams, cephalosporins, carbapenems	Inhibit petidoglycan layer synthesis of cell wall	Production of Beta lactamase to destroy the Beta-lactams	Poole (2004)
2. Aminoglycosides streptomycin, kanamycin, tobramycin, gentamicin	Inhibit bacterial protein synthesis by binding 30S ribosomal subunits	Antibiotic inactivation by plasmid- and transposon encoded modifying enzymes	Kotra *et al.* (2000)
3. Phenicols chloramphenicol florfenicol	Binds reversibly to the teptidyltransferase component of the 50S ribosomal subunit prevent the transpeptidation of peptide chain elongation	Acquisition of plasmids encoding chloramphenicol acetyltransferases (CAT) and which enzymatically inactivate the drug	Falagas *et al.* (2008)
4. Sulfonamides and trimethoprim prontosil, gantrisin, erythromycin-sulfisoxazole	Act competitively inhibiting bacterial modification of para-aminobenzoic acid into dihydrofolate thus interfering with folic acid metabolism	Owing to acquisition of plasmid that encode a drug-resistant dihydropteroate	Chopra (2007)
5. Tetracycline chlortetracycline oxytetracycline, demeclocycline, doxycycline	Binds reversibly to the 30S ribosomal subunits, which blocks the access of aminoacyl t-RNA to the RNA-ribosome complex, to prevent bacterial polypeptide synthesis	Chromosomal mutations affecting outer membrane permeability	Chopra (2007) Falagas *et al.* (2008)
6. Quinolones/fluoroquinolones nalidixic acid	The target is DNA gyrase, essential enzyme for DNA replication	Target gene mutation and removal by efflux pumps	Hooper (2000) Falagas *et al.* (2008)

found that both MRSA and non-MRSA were inhibited at concentrations over 1·35 mg ml^{-1} when the inoculum was 10^5-CFU ml^{-1}. Silver nanoparticles for the treatment for dental caries were used by Espinosa-Cristobal *et al.* (2009). Because *Strep. mutans* is a causal organism of dental caries, they studied the antibacterial effect of silver nanoparticles of three different sizes and reported MIC. They confirmed that silver nanoparticles have strong bactericidal potential against *Strep. mutans*, and this potential is strong when the particle size is diminished.

Nanda and Saravanan (2009) reported the synthesis of silver nanoparticles by aqueous Ag^+ reduction with *Staph. aureus*. These silver nanoparticles were evaluated for their antimicrobial potential against *methicillin*-resistant *Staph. aureus*, methicillin-resistant *Staph. epidermidis* (MRSE), *Strep. pyogenes*, *Salm. typhi* and *Kl. pneumoniae*. They reported the most bactericidal potential against methicillin-resistant *Staph. aureus* followed by *methicillin*-resistant *Staph. epidermidis* and *Strep. pyogenes*, but only moderate activity was observed against *Salm. typhi* and *Kl. pneumoniae*. The authors further reported effective antimicrobial activity of silver nanoparticles against the drug-resistant bacteria MRSA and MRSE, which showed maximum activity against MRSA, followed by MRSE. Humberto *et al.* (2010) determined the antibacterial effect of silver nanoparticles with different concentrations by using bacterial cell viability assay based on luciferase against the erythromycin-resistant *Strep. pyogenes*, ampicillin-resistant *E. coli*, MDR *Ps. aeruginosa* and drug-susceptible strains including *Streptococcus* sp., *E. coli* and *Ps. aeruginosa*. The silver nanoparticles of concentration between 30 and 100 mmol l^{-1} were found to be effective (Humberto *et al.* 2010). Ansari *et al.* (2011) also reported that silver nanoparticles in size range of 5–10 nm showed both bacteriostatic and bactericidal effects against *Staph. aureus*, methicillin-sensitive *Staph. aureus* (MSSA) and MRSA. Colloidal silver nanoparticles having a of size 20–45 nm were synthesized using sol–gel method, their antibacterial activity with MIC against *E. coli, Staph. aureus, C. albicans, B. subtilis, Salm. typhimurium, Ps. aeruginosa* and *Kl. pneumoniae* by the broth microdilution method was determined, and MIC was found to be 2–4 μg ml^{-1} (Lkhagvajav *et al.* 2011). The detailed review of the bactericidal potential of silver nanoparticles against MDR bacteria is described in Table 3.

Table 3 Antibacterial activity of silver nanoparticles against the drug-resistant bacteria

S. No.	Drug-resistant bacteria	References
1.	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Panacek *et al.* (2006)
2.	MRSA and non-MRSA	Ayala-Nunez *et al.* (2009)
3.	*Streptococcus mutans*	
4.	Methicillin-resistant *Staph. aureus*, methicillin-resistant *Staphylococcus epidermidis* (MRSE) and *Streptococcus pyogenes*	Espinosa-Cristobal *et al.* (2009)
5.	MRSA and MRSE	Nanda and Saravanan (2009)
6.	Erythromycin-resistant *Strep. pyogenes*, ampicillin-resistant *Escherichia coli*, multidrug-resistant *Pseudomonas aeruginosa*	Saravanan and Nanda (2010)
7.	*Staph. aureus*, methicillin-sensitive *Staph. aureus* (MSSA), and MRSA	Humberto *et al.* (2010) Ansari *et al.* (2011)
8.	*E. coli, Staph. aureus, Candida albicans, Bacillus subtilis, Salmonella typhimurium, Ps. aeruginosa* and *Klebsiella pneumoniae*	
9.	*Salm. typhi*, *Staph. epidermidis*, *Staph. aureus*, *Ps. aeruginosa*, *Proteus vulgaris*, *E. coli*, *Kl. pneumoniae*	Lkhagvajav *et al.* (2011)
10.	MRSA	Nithya *et al.* (2011)

Antibacterial mode of action

The broad classification of bacteria as Gram-positive and Gram-negative is based upon their different membrane structures and composition. The structural difference between these bacteria is the organization and composition of peptidoglycan layer in the cell wall: peptidoglycan layer is present outside the cytoplasmic membrane. The cell wall of Gram-positive bacteria contains 30-nm-thick peptidoglycan layers, but in case of Gram–negative, 2- to 3-nm peptidoglycan layer is present, which is covered by an outer membrane composed of phospholipids and lipopolysaccharides facing towards the external environment. Although the antimicrobial effect of silver nanoparticles has been extensively studied, their bactericidal mechanism is not clearly understood.

Certain studies showed that silver nanoparticles attacks Gram-negative bacteria by anchoring and penetrating the cell wall, and as a consequence, the leading structural change in the cell membrane takes place and cell permeability increases. Hence, uncontrolled transport through cytoplasmic membrane followed by cell death is the fate (Morones *et al.* 2005; Sondi and Salopek-Sondi 2007). It was also proposed that the antibacterial mechanism of

silver nanoparticles is attributable to the formation of free radicals, which is subsequently followed by free radical-induced membrane damage (Kim *et al.* 2007).

Morones *et al.* (2005) proposed the theory on the basis of the silver ions' tendency of interacting strongly with thiol groups in vital enzymes and phosphorus-containing bases, stating that the damage could be caused by interactions of silver nanoparticles with compounds such as DNA. This interaction may prevent cell division and DNA replication, ultimately leading to cell death. However, there was no DNA damage observed in the experiment carried out (Hwang *et al.* 2008). Shrivastava *et al.* (2008) proposed that silver nanoparticles modulate the phosphotyrosine profile of putative bacterial peptides that can affect cellular signalling, which leads to growth inhibition in bacteria. Hwang *et al.* (2008) studied the stress-specific bioluminescent bacteria and proposed a synergistic toxic effect of the silver nanoparticles and the silver ions produced. They observed that ions move into the cells and lead to the production of reactive oxygen species. Furthermore, owing to the membrane damage caused by nanoparticles, the cells cannot effectively extrude the silver ions and limit their effect (Hwang *et al.* 2008).

A study of the antibacterial activity of silver nanoparticles against Gram-negative bacteria was carried out by Morones *et al.* (2005); which suggested that silver nanoparticles disturb the cell function by attaching to the surface of the cell membrane, penetrating in bacteria, followed by subsequent release of silver ions. Silver nanoparticles are the effective killing agent of broad spectrum of Gram-negative bacteria such as *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella* and *Vibrio*, Gram-positive bacteria such as *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus* and *Streptococcus* and antibiotic-resistant bacteria such as methicillin- and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA) and methicillin-resistant *Ent. faecium* by preventing biofilm formation. Biofilm is a self-secreted extracellular polysaccharide matrix, made up of surface-attached aggregates of micro-organisms. Biofilm acts as an efficient barrier against antimicrobial agents and the host immune system and protect the bacterial colony. It was observed that silver nanoparticles inhibit the formation of biofilms (Percival *et al.* 2007).

Klueh *et al.* (2000) proposed that silver nanoparticles inhibit bacterial growth by inactivating the proteins. Silver atoms bind to thiol groups (−SH) in enzymes, which deactivates the enzymes. Silver alters the function of compounds in cell membrane, which is important in transmembrane energy generation and ion transport, by forming a stable S-Ag bond with the thiol group of the compounds. Silver acts as a catalyst in the formation of disulfide bonds in the reaction of oxygen molecules in the cell and hydrogen atoms of thiol groups (R-S-S-R). Silver catalysed the formation of disulfide bonds responsible for the change in shape and structure of cellular enzymes, which affects their function. It was found that treatment of cells with 900 ppb Ag^{+} solution affects the expression of certain important protein and enzymes such as 30S ribosomal subunit, succinyl co A synthetase, maltose transporter (MalK) and fructose bisphosphate adolase. It was also thought that silver ions bind to the 30S ribosomal subunit, deactivate the ribosome complex and prevent protein translation. The succinyl coenzyme A synthetase, an important enzyme involved in the TCA cycle, found to be downregulated upon treatment with Ag^{+87}. Thus, the proteins, which play important role in aspects of cell, are affected by the silver nanoparticles, leading to bacterial cell death (Yamanaka *et al.* 2005). Klueh *et al.* (2000) hypothesized that bactericidal activity of the silver nanoparticles is attributable to the Ag^{+} ions, which enter the cell and intercalate between the purine and pyrimidine base of DNA. These base pairs showed disturbing effect on the hydrogen bonding between the two antiparallel strands, leading to denaturation of DNA molecule (Klueh *et al.* 2000). All the multiactional behaviours of silver nanoparticles against bacteria are shown in Fig. 1.

Factors influencing the bactericidal effect of silver nanoparticles

Size

Change in reactivity and properties of nanoparticles is attributable to their small size, compared with bulk matter. The smaller the size, the larger the surface-area-to-volume ratio; hence, obviously the bactericidal activity of

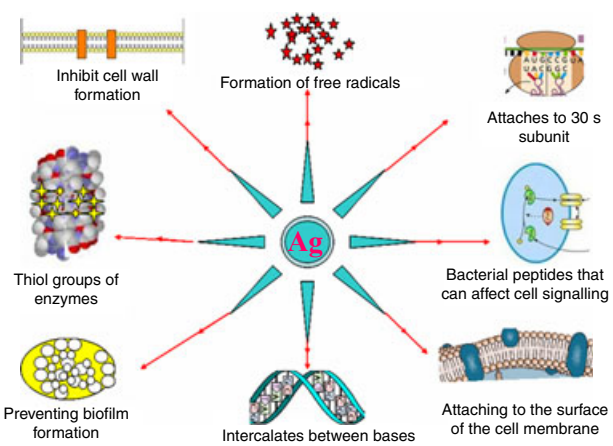

Figure 1 Silver nanoparticles showing multiple bactericidal actions.

silver nanoparticles is affected by the size of the nanoparticles. Depending on the size of the nanoparticles, large surface area comes in contact with the bacterial cells to provide a higher percentage of interaction than bigger particles. Reactivity of nanoparticles is enhanced by the electronic effect produced by the interaction of nanoparticles with bacterial surface, and nanoparticles smaller than 10 nm have high percentage of interaction with bacteria. Thus, the bactericidal effect of silver nanoparticles is size dependent (Morones *et al.* 2005; Raimondi *et al.* 2005). The size dependency of bactericidal potential of nanoparticles was studied by Panacek *et al.* (2006), who reported that the nanoparticles of size 25 nm possessed highest antibacterial activity.

Shape

The bactericidal potential of nanoparticles is also influenced by their shapes, which is shown by studying the bacterial growth inhibition by differentially shaped nanoparticles (Morones *et al.* 2005). In another study, Pal *et al.* (2007) reported the effect of spherical, rod and triangular nanoparticles synthesized by citrate reduction against *E. coli* at different concentrations. It was found that triangular nanoparticles are more active than spherical nanoparticles, which are again more active than rod-shaped nanoparticles against *E. coli* (Pal *et al.* 2007). Thus, antibacterial activities of silver nanoparticles are influenced by shape also.

Concentration

Morones *et al.* (2005) performed the study of bactericidal effect of silver nanoparticles of size 1–100 nm on Gram-negative bacterium *E. coli*. They analysed the interaction of silver nanoparticles with bacteria by growing the bacterial cells up to mid-log phase, measuring OD at 595 nm, studied the effect of different concentrations of silver on bacterial growth and concluded that concentration up to 75 μg ml^{-1} was sufficient for bacterial growth but above that, there was no significant bacterial growth (Morones *et al.* 2005).

Dose

Synthesis of silver nanoparticles of size 10–15 nm and the dose-dependent effect on the Gram-negative and Gram-positive bacteria were studied. They found that activity of silver nanoparticles is dose dependent and silver nanoparticles have noticeable bactericidal activity against Gram-negative bacteria compared to the Gram-positive organisms (Shrivastava *et al.* 2008).

Conclusions

Continuous increase in resistance to drug/antibiotics in human pathogens leads to the re-emergence of MDR pathogens and parasites. Infections caused by such pathogens require a multiple treatment, containing broad-spectrum antibiotics. In fact, these treatments are less effective, more toxic and also expensive. Nanotechnology provides a good platform to overcome the problem of resistance, with the help of the silver nanoparticles. Since the ancient time, antimicrobial efficacy of silver was reported in Ayurveda and homoeopathy. The bactericidal potential can be increased by manipulating the size at nanolevel, leading to increased surface-area-to-volume ratio and also by changing the chemical and physical properties. Silver nanoparticles of size 10–100 nm have strong bactericidal potential against both Gram-positive and Gram-negative bacteria. Therefore, silver nanoparticles having bactericidal potential would be used as powerful weapons against the MDR bacteria such as *Ps. aeruginosa*, ampicillin-resistant *E. coli*, erythromycin-resistant *Strep. pyogenes*, MRSA and VRSA.

References

Abraham, E.P. and Chain, E. (1940) An enzyme from bacteria able to destroy Penicillin. *Nature* **146**, 837.

Ahmad, Z., Pandey, R., Sharma, S. and Khuller, G.K. (2005) Alginate nanoparticles as antituberculosis drug carriers: formulation development, pharmacokinetics and therapeutic potential. *Indian J Chest Dis Allied Sci* **48**, 171–176.

Ansari, M.A., Khan, H.M., Khan, A.A., Malik, A., Sultan, A., Shahid, M., Shujatullah, F. and Azam, A. (2011) Evaluation of antibacterial activity of silver nanoparticles against MSSA and MSRA on isolates from skin infections. *Biol Med* **3**, 141–146.

Atiyeh, B.S., Costagliola, M., Hayek, S.N. and Dibo, S.A. (2007) Effect of silver on burn wound infection control and healing: review of the literature. *Burn* **33**, 139–148.

Ayala-Nunez, N.V., Villegas, H.H.L., Turrent, L.C.I. and Padilla, C.R. (2009) Silver nanoparticles toxicity and bactericidal effect against methicillin resistant *Staphylococcus aureus*: nanoscale does matter. *Nanobiotechnology* **5**, 2–9.

Baker, C., Pradhan, A., Pakstis, L., Pochan, D.J. and Shah, S.I. (2005) Synthesis and antibacterial properties of silver nanoparticles. *J Nanosci Nanotechnol* **2**, 244–249.

Bellinger, C.G. and Conway, H. (1970) Effects of silver nitrate and sulfamylon on epithelial regeneration. *Plast Reconstr Surg* **45**, 582–585.

Bhol, K.C., Alroy, J. and Schechter, P.J. (2004) Anti-inflammatory effect of topical nanocrystalline silver cream on allergic contact dermatitis in a guinea pig model. *Clin Exp Dermatol* **29**, 282–287.

Birla, S.S., Tiwari, V.V., Gade, A.K., Ingle, A.P., Yadav, A.P. and Rai, M.K. (2009) Fabrication of silver nanoparticles by *Phoma glomerata* and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Lett Appl Microbiol* **48**, 173–179.

Biyela, P.T., Lin, J. and Bezuidenhout, C.C. (2004) The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. *Water Sci Technol* **50**, 45–50.

Bonde, S.R., Rathod, D.P., Ingle, A.P., Ade, R.B., Gade, A.K. and Rai, M.K. (2012) Murraya koenigii-mediated synthesis of silver nanoparticles and its activity against three human pathogenic bacteria. *Nanosci Meth* **1**, 25–36.

Butkus, M.A., Labare, M.P., Starke, J.A., Moon, K. and Talbot, M. (2004) Use of aqueous silver to enhance inactivation of coliphage MS-2 by UV disinfection. *Appl Environ Microbiol* **70**, 2848–2853.

Castellano, J.J., Shafii, S.M., Ko, F., Donate, G., Wright, T.E., Mannari, R.J., Payne, W.G., Smith, D.J. *et al.* (2007) Comparative evaluation of silver-containing antimicrobial dressings and drugs. *Int Wound J* **4**, 14–22.

Cebrian, L.E., Sirvent, J.C., Rodriguez, D., Ruiz, M. and Royo, G. (2003) Characterisation of *Salmonella* spp. mutants produced by exposure to various fluoroquinolones. *Int J Antimicrob Agents*, **22**, 134–139.

Chen, X. and Schluesener, H.J. (2008) Nano-silver: a nano-product in medical application. *Toxicol Lett* **176**, 1–12.

Chopra, I. (2007) The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern. *J Antimicrob Chemother* **59**, 587–590.

De Souza, A., Mehta, D. and Leavitt, R.W. (2006) Bactericidal activity of combinations of silver–water dispersion with 19 antibiotics against seven microbial strains. *Curr Sci*, **91**, 926–929.

Duran, N., Marcarto, P.D., De Souza, G.I.H., Alves, O.L. and Esposito, E. (2007) Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. *J Biomed Nanotechnol* **3**, 203–208.

Espinosa-Cristobal, L.F., Martinez-Castanon, G.A., Martinez-Martinez, R.E., Loyola-Rodriguez, J.P., Patino-Marin, N., Reyes-Macias, J.F. and Ruiz, F. (2009) Antibacterial effect of silver nanoparticles against *Streptococcus mutans*. *Mater Lett* **63**, 2603–2606.

Falagas, M.E., Grammatikos, A.P. and Michalopoulos, A. (2008) Potential of old-generation antibiotics to address current need for new antibiotics. *Expert Rev Anti Infect Ther* **6**, 593–600.

Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N. and Kim, J.O. (2000) Mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mater Res* **52**, 662–668.

Gade, A., Gaikwad, S., Tiwari, V., Yadav, A., Ingle, A. and Rai, M. (2010) Biofabrication of silver nanoparticles by *Opuntia ficus-indica*: in vitro antibacterial activity and study of the mechanism involved in the synthesis. *Curr Nanosci* **6**, 370–375.

Gajbhiye, M., Kesharwani, J., Ingle, A., Gade, A. and Rai, M. (2009) Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. *Nanomedicine* **5**, 382–386.

Geethalakshmi, R. and Sarada, D.V.L. (2010) Synthesis of plant-mediated silver nanoparticles using Trianthema decandra extract and evaluation of their anti microbial activities. *Int J Eng Sci Technol* **2**, 970–975.

Gemmell, C.G., Edwards, D.I. and Frainse, A.P. (2006) Guidelines for the prophylaxis and treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the UK. *J Antimicrob Chemother* **57**, 589–608.

Gong, P., Li, H., He, X., Wang, K., Hu, J., Tan, W., Tan, S. and Zhang, X.Y. (2007) Preparation and antibacterial activity of Fe_3O_4@Ag nanoparticles. *Nanotechnology* **18**, 604–611.

Govindaraju, K., Tamilselvan, S., Kiruthiga, V. and Singaravelu, G. (2010) Biogenic silver nanoparticles by *Solanum torvum* and their promising antimicrobial activity antimicrobial activity of silver nanoparticles. *J. Biopest.* **3**, 394–399.

Gu, H., Ho, P.L., Tong, E., Wang, L. and Xu, B. (2003) Presenting vancomycin on nanoparticles to enhance antimicrobial activities. *Nano Lett* **3**, 1261–1263.

Hernane, S.B., Regiani, T., Marques, R.F.C., Lustri, W.R., Messaddeq, Y. and Ribeiro, S.J.L. (2011) Antimicrobial bacterial cellulose-silver nanoparticles composite membranes. *J. Nanomate.* **721**, 631–639.

Hooper, D.C. (2000) Mechanisms of action and resistance of older and newer fluoroquinolones. *Clin Infect Dis* **31**, S24–S28.

Humberto, H., Lara, V., Ayala-Nunez, N.V., Carmen, L.D., Ixtepan, T. and Cristina, R.P. (2010) Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. *World J Microbiol Biotechnol* **26**, 615–621.

Hwang, E.T., Lee, J.H., Chae, Y.J., Kim, Y.S., Kim, B.C., Sang, B. and Gu, M.B. (2008) Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria. *Small* **4**, 746–750.

Ingle, A., Gade, A., Pierrat, S., Sonnichsen, C. and Rai, M. (2008) Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. *Curr Nanosci* **4**, 141–144.

Jun, J., Yuan-Yuan, D., Shao-hai, W., Shao-feng, Z. and Zhongyi, W. (2007) Preparation and characterization of antibacterial silver-containing nanofibers for wound dressing applications. *J US China Med Sci* **4**, 52–54.

Kawahara, K., Tsuruda, K., Morishita, M. and Uchida, M. (2000) Antibacterial effect of silver zeolite on oral bacteria under anaerobic condition. *Dent Mater* **16**, 452–455.

Kim, J.S., Kuk, E., Yu, K.N., Kim, J.H., Park, S.J., Lee, H.J., Jeong, D.H. and Cho, M.H. (2007) Antimicrobial effects

of silver nanoparticles. *Nanomed Nanotechnol Biol Med* **3**, 95–101.

Ki-Young, N. (2011) In vitro antimicrobial effect of the tissue conditioner containing silver nanoparticles. *J Adv Prosthodont* **3**, 20–24.

Klueh, U., Wagner, V., Kelly, S., Johnson, A. and Bryers, J.D. (2000) Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. *J Biomed Mater Res* **53**, 621–631.

Knetsch, M.L.W. and Leo, H.K. (2011) New strategies in the development of antimicrobial coatings: the example of increasing usage of silver and silver nanoparticles. *Polymers* **3**, 340–366.

Kotra, L.P., Haddad, J. and Mobashery, S. (2000) Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob Agents Chemother* **44**, 3249–3256.

Landsdown, A.B.G. (2002) Silver I: its antibacterial properties and mechanism of action. *J Wound Care* **11**, 125–138.

Lkhagvajav, N., Yasab, I., Çelikc, E., Koizhaiganova, M. and Saria, O. (2011) Antimicrobial activity of colloidal silver nanoparticles prepared by sol gel method. *Dig J Nanomater Biostruct* **6**, 149–154.

Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun, H., Kwong-Hang, P., Chiu, T.J. *et al.* (2006) Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *J Proteome Res* **5**, 916–924.

Maneerung, T., Tokura, S. and Rujiravanit, R. (2008) Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. *Carbohydr Polym* **72**, 43–51.

Maple, P.A.C., Hamilton-Miller, J.M.T. and Brranfltt, W. (1992) Comparison of the in-vitro activities of the topical antimicrobials azelaic and, nitroforazone, silver sulphadiazine and mupirocin against methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* **29**, 661–668.

Marcato, P.D. and Duran, N. (2008) New aspects of nanopharmaceutical delivery systems. *J Nanosci Nanotechnol* **8**, 2216–2229.

Matsumura, Y., Yoshikata, K., Kunisaki, S.I. and Tsuchido, T. (2003) Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Appl Environ Microbiol* **69**, 4278–4281.

Mcdonnell, G. and Russell, D. (1999) Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* **12**, 147–179.

Morones, J.R., Elechiguerra, J.L., Camacho, A. and Ramirez, J.T. (2005) The bactericidal effect of silver nanoparticles. *Nanotechnology* **16**, 2346–2353.

Moyer, C.A., Brentano, L., Gravens, D.L., Margraf, H.W. and Monafo, W.W. (1965) Treatment of large human burns with 0.5% silver nitrate solution. *Arch Surg* **90**, 812–867.

Namasivayam, S.K.R., Ganesh, S. and Avimanyu, B. (2011) Evaluation of anti-bacterial activity of silver nanoparticles synthesized from *Candida glabrata* and *Fusarium oxysporum*. *Int J Med Res* **1**, 131–136.

Nanda, A. and Saravanan, M. (2009) Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE. *Nanomedicine* **5**, 452–456.

Nithya, G.N., Shepangam, H. and Balaji, S. (2011) Biosynthesis of silver nanoparticle and its antibacterial activity. *Arch Appl Sci Res* **3**, 377–380.

Oberdorster, G., Maynard, A., Donaldson, K., Castranova, V., Fitzpatrick, J. and Ausman, K. (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Part Fibre Toxicol* **2**, 38–43.

Pal, S., Tak, Y.K. and Song, J.M. (2007) Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Appl Environ Microbiol* **27**, 1712–1720.

Panacek, A., Kvitek, L., Prucek, R., Kolar, M., Vecerova, R., Pizurova, N., Sharma, V.K., Nevecna, T. *et al.* (2006) Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. *J Phys Chem* **110**, 16248–16253.

Percival, S.L., Bowler, P.G. and Dolman, J. (2007) Antimicrobial activity of silver-containing dressings on wound microorganisms using an in vitro biofilm model. *Int Wound J*, **4**, 186–191.

Poole, K. (2004) Resistance to beta-lactam antibiotics. *Cell Mol Life Sci* **61**, 2200–2223.

Rai, M., Yadav, A. and Gade, A. (2009) Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv* **27**, 76–83.

Raimondi, F., Scherer, G.G., Kotz, R. and Wokaun, A. (2005) Nanoparticles in energy technology: examples from electrochemistry and catalysis. *Angew Chem Int Ed Engl* **44**, 2190–2209.

Saravanan, M. and Nanda, A. (2010) Extracellular synthesis of silver bionanoparticles from *Aspergillus clavatus* and its antimicrobial activity against MRSA and MRSE. *Coll Surfaces B Biointerfaces* **77**, 214–218.

Shahverdi, A.R., Fakhimi, A., Shahverdi, H.R. and Minaian, S. (2007) Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomedicine* **3**, 168–171.

Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P. and Dash, D. (2008) Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnol.* **18**, 103–112.

Silvestry-Rodriguez, N., Sicairos-Ruelas, E.E., Gerba, C.P. and Bright, K.R. (2007) Silver as a disinfectant. *Rev Environ Contam Toxicol* **191**, 23–45.

Singh, R. and Singh, N.H. (2011) Medical applications of nanoparticles in biological imaging, cell labeling, antimicrobial agents, and anticancer nanodrugs. *J Biomed Nanotechnol* **7**, 489–503.

Slonczewski, J. and Foster, J. (2009) *Microbiology: An Evolving Science*. New York: W.W. Norton & Company.

Sondi, I. and Salopek-Sondi, B. (2007) Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. *J Colloid Interface Sci* **275**, 77–82.

Spacciapoli, P., Buxton, D., Rothstein, D. and Friden, P. (2001) Antimicrobial activity of silver nitrate against periodontal pathogens. *J Periodontal Res* **36**, 108–113.

Stefan, M., Lucian, H., Marius, M., Daniela, P., Irina, G., Romeo-Iulian, O., Simona, D. and Viorel, M. (2011) Enhanced antibacterial effect of silver nanoparticles obtained by synthesis in poly amide-hydroxyurethane media. *J Mater Sci Mater Med* **22**, 789–796.

Sun, R.W., Chen, R., Chung, N.P., Ho, C.M., Lin, C.L. and Che, C.M. (2005) Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. *Chem Commun (Camb)* **40**, 5059–5061.

Susan, W.P.W., Willie, J.J.M., Carla, A.H., Werner, I.H., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I. *et al.* (2009) Nano-silver-a review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology* **3**, 109–138.

Tenover, F.C. (2006) Mechanisms of antimicrobial resistance in bacteria. *Am J Med* **119**, S3–S10.

Tian, J., Wong, K.K., Ho, C.M., Lok, C.N., Yu, W.Y., Che, C.M., Chiu, J.F. and Tam, P.K. (2007) Topical delivery of silver nanoparticles promotes wound healing. *Chem Med Chem* **2**, 129–136.

Warheit, D.B., Borm, P.J., Hennes, C. and Lademann, J. (2007) Testing strategies to establish the safety of nanomaterials: conclusions of an ECETOC workshop. *Inhal Toxicol* **19**, 631–643.

Webb, G.F., D'Agata, E.M., Magal, P. and Ruan, S. (2005) A model of 499 antibiotic-resistant bacterial epidemics in hospitals. *Proc Nat Acad Sci* **102**, 13343–13348.

Yamanaka, M., Hara, K. and Kudo, J. (2005) Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Appl Environ Microbiol* **71**, 7589–7593.