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ORIGINAL PAPER

Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria

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Abstract Infections caused by drug-resistant microorganisms result in significant increases in mortality, morbidity, and cost related to prolonged treatments. The antibacterial activity of silver nanoparticles against some drug-resistant bacteria has been established, but further investigation is needed to determine whether these particles could be an option for the treatment and prevention of drug-resistant microbial infections. Hence, we challenged different drug-resistant pathogens of clinical importance (multidrug-resistant *Pseudomonas aeruginosa*, ampicillin-resistant *Escherichia coli* O157:H7 and erythromycin-resistant *Streptococcus pyogenes*) with a suspension of silver nanoparticles. By means of a luciferase-based assay, it was determined that silver nanoparticles (1) inactivate a panel of drug-resistant and drug-susceptible bacteria (Gram positive and Gram negative), (2) exert their antibacterial activity through a bactericidal rather than bacteriostatic mechanism, and (3) inhibit the bacterial growth rate from the time of first contact between the bacteria and the nanoparticles. Additionally, strains with a resistant phenotype to silver nanoparticle were developed and used to explore the bactericidal mode of action of silver nanoparticles. Through a Kirby–Bauer test, it was shown that silver nanoparticles' general mechanism of bactericidal action is based on inhibition of cell wall synthesis, protein synthesis mediated by the 30s ribosomal subunit, and nucleic acid synthesis. Our data suggest that silver nanoparticles are effective broad-spectrum biocides against a variety of drug-resistant bacteria, which makes them a potential candidate for use in pharmaceutical products and medical devices that may help to prevent the transmission of drug-resistant pathogens in different clinical environments.

Keywords Drug-resistant bacteria · Silver nanoparticles · Silver resistance · Broad-spectrum agent · Bactericidal agent · Nanobiotechnology

Introduction

Drug-resistant bacteria are emerging pathogens whose resistance profiles present a major challenge for containing their spread and their impact on human health. Currently, over 70% of bacterial nosocomial infections in the United States are resistant to one or more of the antibiotics traditionally used to eliminate them. People who become infected with drug-resistant microorganisms usually spend more time in the hospital and require a form of treatment that uses two or three different antibiotics and is less effective, more toxic, and more expensive (Webb et al. 2005).

Nanotechnology offers opportunities to re-explore the biological properties of already known antimicrobial compounds by manipulating their size to alter the effect. Silver has long been known for its antimicrobial properties, but its medical applications declined with the development of antibiotics. Nonetheless, Credés prophylaxis for gonococcal *ophthalmia neonatorum* remained the standard of care in

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many countries until the end of the twentieth century (Hoyme 1993). Currently, silver sulfadiazine is listed by the World Health Organization as an essential anti-infective topical medicine (World Health Organization 2007). Since silver works as a bulk material, the use of nano-size silver may also be appealing.

Different studies have established the bactericidal effect of nanosilver against Gram negative and Gram positive bacteria, but the bactericidal mechanism of this compound has not been clearly elucidated. Morones et al. (2005) defined the antibacterial activity of silver nanoparticles against four types of Gram negative bacteria, *E. coli*, *V. cholera*, *P. aeruginosa* and *S. typhus*, and suggested that silver nanoparticles attach to the surface of the cell membrane and disturb its function, penetrate bacteria, and release silver ions (Morones et al. 2005). Other groups have worked with Gram positive bacteria, such as *Staphylococcus aureus* (Shrivastava et al. 2007). Furthermore, the antiviral capability of silver nanoparticles against human immunodeficiency virus type 1 (Elechiguerra et al. 2005) and hepatitis B virus (Lut et al. 2008) has been established.

The development of nanosilver products is expanding. Nowadays, nanosilver is found in clothing, food containers, wound dressings, ointments, implant coatings, and other items; some nanosilver applications have received approval from the US Food and Drug Administration (Dunn and Edwards-Jones 2004). Whether silver nanoparticles are an option to confront the transmission of and infection by pathogenic drug-resistant bacteria remains to be determined.

To explore the biocidal properties of silver nanoparticles against these drug-resistant pathogens, we challenged clinical isolates of multidrug-resistant *Pseudomonas aeruginosa*, ampicillin-resistant *E. coli* O157:H7 and erythromycin-resistant *Streptococcus pyogenes* and drug-susceptible strains of the same pathogens with a suspension of silver nanoparticles. By means of a luciferase-based assay, the antibacterial activity of the silver nanoparticles was assessed by determining the minimal inhibitory concentration (MIC), the minimal bactericidal concentration (MBC) and the MBC/MIC ratio; time-kill assays were also used. Furthermore, silver nanoparticle-resistant strains were developed and used to explore the bactericidal mode of action of silver nanoparticles against these bacteria.

Materials and methods

Silver nanoparticles and bacterial strains

A stock solution of commercially manufactured 100 nm silver nanoparticles (Sigma–Aldrich, St. Louis, MO) was prepared in culture media. The subsequent dilutions were made in Luria–Bertani broth.

Methicillin-resistant *S. aureus*, ampicillin-resistant *Escherichia coli* O157:H7, multidrug-resistant *P. aeruginosa*, and drug-susceptible *S. aureus*, *P. aeruginosa* and *Escherichia coli* were cultured at 35°C on Mueller–Hinton agar. Erythromycin-resistant *S. pyogenes* and *Streptococcus* sp. Were cultured at 35°C on blood agar.

MIC and MBC determination

MIC and the MBC were determined by a microdilution method, using Luria–Bertani broth (Sigma–Aldrich) and an inoculum of 2.5×10^5 CFU/mL. Bacteria were incubated with serial twofold dilutions of silver nanoparticles, and the effect on cell viability was measured after 24 h. The MIC value corresponded to the concentration that inhibited 99% of bacterial growth and the MBC value corresponded to the concentration where 100% of the bacterial growth was inhibited, compared to the positive control (no treatment). Bacterial cell viability was measured with the BacTiter-Glo™ Microbial Cell Viability Assay from Promega (Madison, WI), a luciferase based assay that quantifies ATP produced by metabolically active cells. All assays were performed in the Biosafety Laboratory Level 3 (BSL-3) at the Universidad Autonoma de Nuevo Leon.

Time-kill assays

Bacterial growth after treatment was measured by quantifying cell viability at 0, 2, 4, 6, and 24 h after incubation with different concentrations of silver nanoparticles (0.0, 6.25, 12.5, 25.0 and 50.0 mM). The growth inhibition percentage was obtained with respect to the positive control. Bacterial cell viability was measured with the BacTiter-Glo™ Microbial Cell Viability Assay from Promega.

Silver nanoparticle-resistant strains

A strain of methicillin-resistant *S. aureus* (MRSA), drug-susceptible *S. aureus*, ampicillin-resistant *E. coli* O157:H7, and multidrug-resistant *P. aeruginosa* were serially transferred on Mueller–Hinton agar containing graded concentrations of silver nanoparticles (12.5–200 mM) until they could grow in the presence of concentrations near or over the MIC. In the case of MRSA and drug-susceptible *S. aureus*, the MIC reference value that was used was obtained from other publications from our group (in press). These strains were labeled as $AgNP^R$ (silver nanoparticle resistant) whereas the parent strain was designed as $AgNP^S$ (silver nanoparticle susceptible). Both strains were maintained on nutrient agar with or without silver nanoparticles for $AgNP^S$ and $AgNP^R$, respectively (Gupta et al.1992).

To confirm the resistance to silver nanoparticles, $AgNP^S$ and $AgNP^R$ strains were both cultured on a 50 mM silver nanoparticle agar plate. As expected, only the $AgNP^R$ strain was able to grow in these conditions. Once the resistant status to silver nanoparticles was defined, a resistance profile for a panel of antibiotics was determined for each strain using the Kirby–Bauer test with Bio-Rad multidiscs (Hercules, CA) and NCCLS parameters. The sensitivity to each antibiotic was proportional to the diameter of the inhibition halo.

Statistical analysis

Minimal inhibitory concentration, MBC and the Kirby–Bauer tests were performed in triplicate, and the results are expressed as means ± the standard errors of the means. A Student's *t*-test was used to compare these results. *P* values lower than 0.05 were considered significant. SigmaPlot 10.0 was used to create the figures.

Results

Bactericidal activity of silver nanoparticles

A luciferase-based bacterial cell viability assay was used to determine the bactericidal effect of different concentrations of silver nanoparticles on erythromycin-resistant *S. pyogenes*, ampicillin-resistant *E. coli* O157:H7, multidrug-resistant *P. aeruginosa,* and three drug-susceptible strains (Table 1). The minimum inhibitory concentrations and minimum bactericidal concentrations of silver nanoparticles ranged between 30 and 100 mM, respectively. The MBC/MIC ratio is a parameter that reflects the bactericidal capacity of the analyzed compound. In our study, silver nanoparticles exerted a bactericidal effect against the six bacterial strains because the MBC/MIC ratio values were lower than 1.2. As seen in Table 1, there was no significant difference between the bactericidal effects of silver nanoparticles on drug-resistant and non-drug-resistant microorganisms. The bactericidal activity was not affected by the cell membrane structure, given that both Gram positive and Gram negative bacteria were inhibited.

Effect of Ag-NPs on bacterial growth

The bactericidal activity of different concentrations of silver nanoparticles (0.0, 6.25, 12.5, 25.0 and 50.0 mM) was compared among the different drug-resistant strains using time-kill assays. The time-kill assays were used to analyze post-treatment bacterial viability and to define the minimum time necessary to reach an inhibitory or bactericidal effect. Since no significant difference was found between the bactericidal effects of silver nanoparticles on the different bacteria, drug-susceptible strains were not used in this assay.

Silver nanoparticle treatment affected bacterial growth to different extents (Fig. 1). The effect was proportional to the dose since 50.0 mM was the most effective treatment (the bacterial population did not recover) and 6.25 was the least effective. Although 6.25 mM is considerably under the MIC–MBC range of silver nanoparticles, the bacterial population did not reach normal levels of growth after 24 h of incubation.

No minimum time of exposure to silver nanoparticles is needed to achieve an inhibitory effect. At the initial time point (0 h), 50.0 mM of silver nanoparticles inhibited most of the bacterial populations (data not shown). After 24 of incubation, no significant recovery was observed since the same nanosilver concentration inhibited 99.7% of erythromycin-resistant *S. pyogenes* (Fig. 1a), 95.7% of ampicillin-resistant *E. coli* O157:H7 (Fig. 1b) and 92.8% of multidrug-resistant *P. aeruginosa* (Fig. 1c).

Table 1 MICs and MBCs for individual strains

	MIC (mM)[a]	MBC (mM)[a]	MBC/MIC ratio
Drug-resistant bacteria			
Erythromycin-resistant *S. pyogenes*	66.7 (±16.7)	66.7 (±16.7)	1.0
Ampicillin-resistant *E. coli* O157:H7	83.3 (±16.7)	83.3 (±16.7)	1.0
Multidrug-resistant *P. aeruginosa*	83.3 (±16.7)	100.0 (±0.0)	1.2
Average	79.4	83.3	1.1
Drug-susceptible bacteria			
Streptococcus sp.	29.2 (±11.0)	29.2 (±11.0)	1.0
Escherichia coli	83.3 (±16.7)	83.3 (±16.7)	1.0
P. aeruginosa	83.3 (±16.7)	83.3 (±16.7)	1.0
Average	65.3	65.3	1.0
All	71.5	74.3	1.1

MIC minimal inhibitory concentration, *MBC* minimal bactericidal concentration

[a] Mean ± standard error of the mean

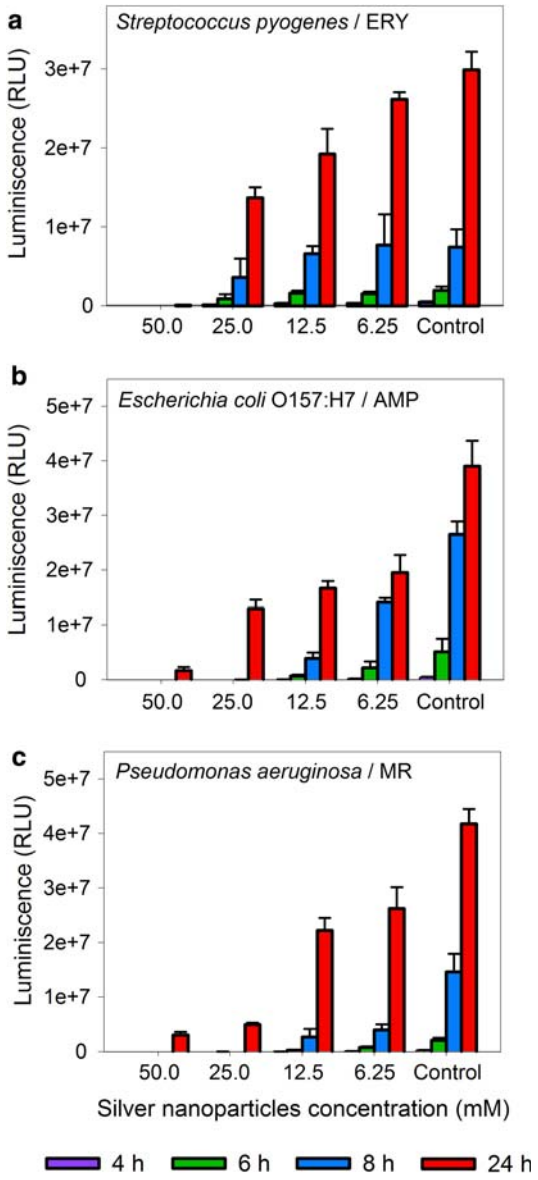

Fig. 1 Time-kill assays of drug-resistant bacteria. Viability results were measured by a luciferase-based assay of **a** erythromycin-resistant *S. pyogenes,* **b** ampicillin-resistant *E. coli* O157:H7, and **c** multidrug-resistant *P. aeruginosa* against different concentrations of silver nanoparticles. The measurements were made at 4, 6, 8, and 24 h post-treatment. The assay was performed in triplicate and the data points represent the mean ± SEM. *RLU* relative light units, *ERY* erythromycin, *AMP* ampicillin, *MR* multidrug-resistant

Mode of bactericidal action of silver nanoparticles against drug-resistant bacteria

Once the bactericidal effect of silver nanoparticles against distinct drug-resistant bacteria was defined, the mode of action was examined by analyzing silver nanoparticle-resistant strains. For this study, MRSA and drug-susceptible *S. aureus* were used as model Gram positive microorganisms, and multidrug-resistant *P. aeruginosa* and ampicillin-resistant *E. coli* O157:H7 were used as Gram negative models. These bacteria were serially transferred on agar prepared with increasing concentrations of silver nanoparticles until they reached a concentration that inhibited colony growth. After 3 weeks, MRSA was able to grow at a silver nanoparticle concentration of 100 mM, drug-susceptible *S. aureus* at 200 mM, multidrug-resistant *P. aeruginosa* at 75 mM, and ampicillin-resistant *E. coli* O157:H7 at 75 mM. These strains were labeled as $AgNP^R$ whereas the parent susceptible strains were designed as $AgNP^S$.

The colony morphology of the $AgNP^R$ strains was considerably different than the $AgNP^S$ strains grown in agar without nanoparticles. As seen in Fig. 2a–c, $AgNP^R$ colonies acquired a grayish silver color after growing on silver nanoparticle agar. These colonies presented a circular or irregular form, smooth surface, and mucoid or butyrous texture. The grayish color indicates that $AgNP^R$ strains were able to take in silver nanoparticles from the substrate, absorb them, accumulate them inside the cell, and block the bactericidal effect of the silver nanoparticles.

To define the differences between $AgNP^R$ and $AgNP^S$ strains, antibiograms (Kirby–Bauer tests) were performed. All $AgNP^R$ strains presented modified resistance profiles compared to the $AgNP^S$ strains (Fig. 3a–d), which indicates that the acquisition of silver nanoparticle resistance causes changes in structures that also participate in the response to common antibiotics. Basically, the antibiotics that showed altered effects in $AgNP^R$ strains include antibiotics that inhibit wall synthesis (Fig. 3a, d), protein synthesis mediated by the 30s ribosomal subunit (Fig. 3b), or nucleic acids synthesis (Fig. 3c). As shown in Fig. 3, variations in the response to antibiotics included both increases and decreases in sensitivity. Sensitivity to cefuroxime (cephalosporin) for the drug-susceptible *S. aureus* $AgNP^R$ strain was considerably decreased, resulting in a change of status from Susceptible (S) to Resistant (R) according to NCCLS parameters.

Discussion

In the current study, we aimed to determine through different in vitro assays the antibacterial properties of silver nanoparticles against drug-resistant bacteria, infectious agents that represent a constant threat in hospital and community environments. To achieve this goal, we challenged three Mexican clinical isolates classified as resistant to one or more antibiotics with different concentrations of a nanosilver suspension and described the effect on bacterial cell viability and growth rate. To gain a more complete

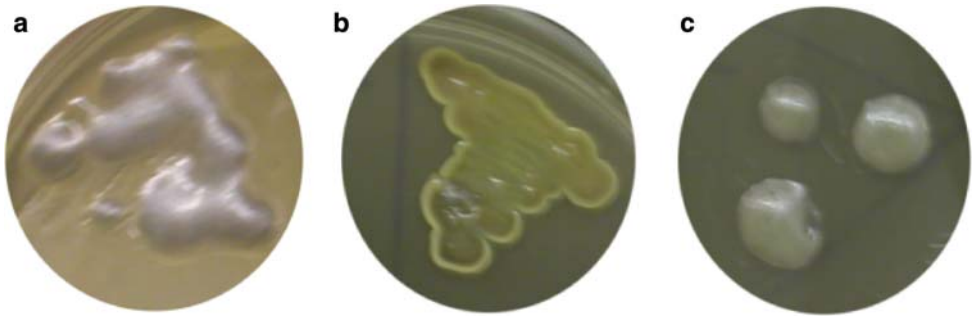

Fig. 2 AgNP^R colonies of drug-susceptible *S. aureus* grown at 20 mM (**a**) and 25 mM (**b**), multidrug-resistant *P. aeruginosa* grown at 25 mM (**c**). All strains were serially transferred on Mueller–Hinton agar containing graded concentrations of silver nanoparticles until a concentration near or over the MIC was reached on which the bacteria could grow

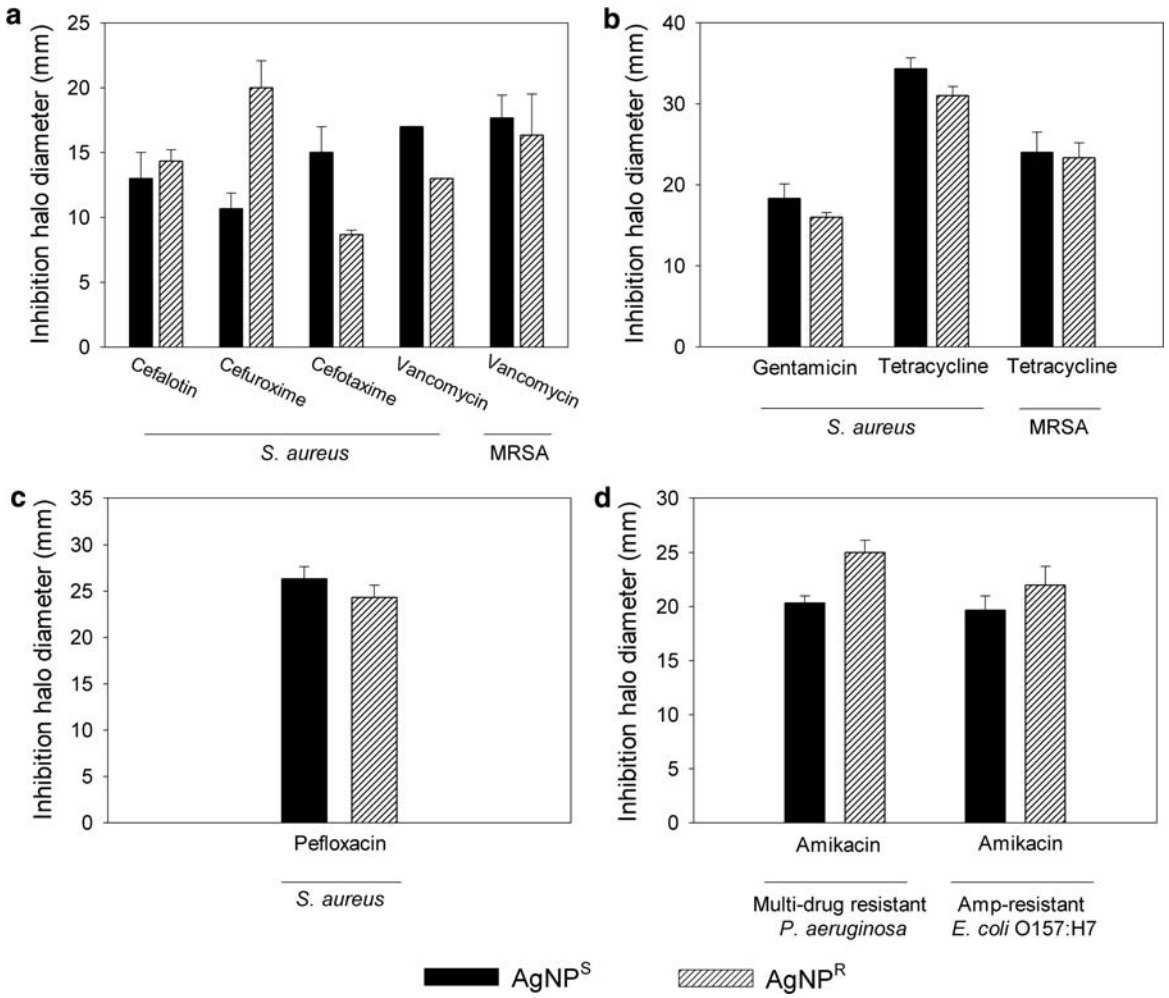

Fig. 3 Characterization of AgNP^R strains with respect to their resistance to a panel of different antibiotics. An antibiotic sensitivity test (Kirby–Bauer) was performed for AgNP^R strains of MRSA, drug-susceptible *S. aureus,* multidrug-resistant *P. aeruginosa*, and ampicillin-resistant *E. coli* O157:H7. The sensitivity to each antibiotic was proportional to the diameter of the inhibition halo. Gram positive AgNP^R bacteria altered their sensitivity to antibiotics that **a** inhibit wall synthesis, **b** inhibit protein synthesis mediated by the 30s ribosomal subunit, and **c** inhibit nucleic acid synthesis. Gram negative bacteria altered their sensitivity to antibiotics that **d** inhibit protein synthesis. The assay was performed in triplicate and the *bars* represent the mean ± SEM

understanding and to attempt a preliminary approach to determining the mechanism of inhibition of silver nanoparticles, a comparison was made between multidrug-resistant strains and drug-susceptible strains of the same species and between Gram negative and Gram positive bacteria.

For all strains, the average ratio of the minimum bactericidal concentration to the minimum inhibitory concentration indicated that silver nanoparticles have a bactericidal rather than bacteriostatic effect on the tested bacteria. In theory, a bactericidal agent is preferred clinically because bacterial killing should produce a faster resolution of the infection, improve clinical outcome, and reduce the likelihood of the emergence of resistance and the spread of infection. If pathogens are killed rather than inhibited, resistance mutations that might otherwise emerge as the result of antibiotic pressure are eliminated (French 2006).

No significant differences in bactericidal activity were found among the different compared groups (drug-resistant vs. susceptible, Gram positive vs. negative), which suggests that silver nanoparticles are broad spectrum antibacterial agents. These results further agree with previous findings by other research teams, where it was proven that silver nanoparticles exert the same effect on Gram positive and Gram negative strains (Kong and Jang 2008; Petica et al. 2008). Shrivastava et al. postulated that Gram negative bacteria are less susceptible to silver nanoparticles because the positive charges of the silver nanoparticles interact with the Gram negative lipopolysaccharide with more affinity than with the Gram positive cellular wall, which is thought to have fewer interaction sites with positive charges (Shrivastava et al. 2007). However, in our results *E. coli* and *P. aeruginosa* were less susceptible (although not significantly) to silver nanoparticles, so lipopolysaccharide might not be a structure that makes bacterial cells more receptive to the effect of silver nanoparticles. Instead, lipopolysaccharide might trap and block the positive charges of silver nanoparticles and make Gram negative bacteria less susceptible to them. Indeed, silver nanoparticles attach to the surface of the cell membrane and disturb its function, penetrate bacteria, and release silver ions. Sondi and Salopek-Sondi (2004) and Lok et al. (2006) found that silver nanoparticles target the bacterial membrane, leading to a dissipation of the proton motive force (Lok et al. 2007; Sondi and Salopek-Sondi 2004). Consequently silver nanoparticles need to reach the cell membrane to achieve an antibacterial effect.

The fact that the drug-resistant and drug-susceptible strains were affected by silver nanoparticles in the same manner indicates that the drug-resistant proteins that give bacteria the capacity to avoid antibiotics do not affect the efficacy of nanosilver.

One of the principal elements of bacteria's infectivity is their rapid reproduction time, a characteristic that could be a good target for impeding a viable infection. As shown by time-kill assays, silver nanoparticles were effective in inhibiting bacterial growth in a dose and time dependent manner. Yamanaka, et al. (2005) found that silver ions require about 14 h to reduce an *E. coli* population from 10^7 to 10^1 CFU/mL. As mentioned by Pal et al. (2007) the activity of nanoparticles might by similar to that of silver ions.

By using $AgNP^R$ strains (Fig. 2), the mode of the bactericidal action of silver nanoparticles was explored. Resistance to silver nanoparticles implies changes in the inhibited cellular target(s). Therefore, if a change occurs in a protein or pathway targeted by an antibiotic, the bacterial sensitivity to this antibiotic is modified. To characterize the $AgNP^R$ strains with respect to their resistance to a panel of different antibiotics, a sensitivity test (Kirby–Bauer) was performed. For the $AgNP^R$ strains of MRSA, drug-susceptible *S. aureus,* multidrug-resistant *P. aeruginosa,* and ampicillin-resistant *E. coli* O157:H7, the Kirby–Bauer test showed an altered antibiotic resistance phenotype (Fig. 3), indicating that silver nanoparticles and these antibiotics share a common target in the bacteria.

Gram positive $AgNP^R$ strains exhibited altered responses to different cephalosporins, glycopeptides, aminoglycosides, tetracilins, and fluoroquinolones (Fig. 3a–c), but Gram negative $AgNP^R$ strains only exhibited altered responses to Amikacin, an aminoglycoside (Fig. 3d). Since aminoglycosides inhibit protein synthesis by blocking the 30s ribosomal subunit, silver nanoparticles may inhibit this pathway while exerting their bactericidal activity against Gram negative bacteria. On the other hand, silver nanoparticles target protein synthesis, nucleic acid synthesis, and Gram positive cell wall synthesis, which explains why these bacteria were more susceptible (although not significantly more) to silver nanoparticles (Table 1). Gram negative bacteria tend to be less susceptible to the effect of compounds that act on the cell wall, including B-lactam antibiotics.

Our findings suggest that the mode of action of silver nanoparticles is similar to that of silver ions, which complex with electron donor groups containing sulfur, oxygen or nitrogen atoms that are normally present as thiols or phosphates (McDonnell 2007) on amino acids and nucleic acids. Like silver nanoparticles, silver ions also exert their activity through a broad range of mechanisms, including denaturing the 30s ribosome subunit, suppressing the expression of enzymes and proteins essential to ATP production (Yamanaka et al. 2005), inhibiting respiratory enzymes thereby inducing the production of reactive oxygen species (Matsumura et al. 2003; Yamanaka et al. 2005), binding and dimerizing RNA and DNA (Rai et al. 2009), and destabilizing and disrupting the outer membrane (Lok et al. 2006).

Reports of silver ion-resistant strains have also indicated a modification in the response to antibiotics, such as the acquisition of resistance to mercuric chloride, ampicillin, chloramphenicol, tetracycline, streptomycin and sulfonamides (Chopra 2007).

Interestingly, after subculturing the $AgNP^R$ strains in the absence of silver nanoparticles, the resistance phenotype was lost (data not shown). Other clinical studies identified silver resistance in members of the *Enterobacteriaceae*, and the resistance phenotype was also unstable in the absence of silver selective pressure. According to Chopra (2007), this instability of the phenotype could reflect reversions of the chromosomal mutations conferring silver resistance, especially if they impose fitness costs, or it may reflect the loss of plasmids encoding resistance (Chopra 2007).

Besides their bactericidal activity and immediate antibacterial effect against a wide variety of drug-resistant bacteria, silver nanoparticles have particular characteristics provided by the silver itself. This noble metal tends to induce low bacterial resistance (Ip et al. 2006) and has low toxicity and minimal side effects when ingested since at most 2–4% is retained in tissues after absorption by the body. A notable health effect has been argyria, an irreversible pigmentation of the skin that is mostly an aesthetic concern (Drake and Hazelwood 2005).

The bactericidal activity of silver nanoparticles against multidrug-resistant bacteria could be used in conjunction with advances in impregnation techniques and polymer technology to expand the range of applications of these nanoparticles in the preservation of food, disinfection of medical supplies and equipment, and decontamination of the surfaces of items such as toys and kitchenware (Matsumura et al. 2003).

The data presented here are novel in that they prove that silver nanoparticles are effective bactericidal agents regardless of the drug-resistance mechanisms that exist in multidrug-resistant *P. aeruginosa*, ampicillin-resistant *E. coli* O157:H7 and erythromycin-resistant *S. pyogenes* and show the importance of silver nanoparticles in the nosocomial and community environment. Therefore, silver nanoparticles can be recommended as an effective broad-spectrum bactericidal agent.

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References

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

Drake PL, Hazelwood KJ (2005) Exposure-related health effects of silver and silver compounds: a review. Ann Occup Hyg 49:575–585

Dunn K, Edwards-Jones V (2004) The role of Acticoat with nanocrystalline silver in the management of burns. Burns 30(Suppl 1):S1–S9

Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH, Yacaman MJ (2005) Interaction of silver nanoparticles with HIV-1. J Nanobiotechnology 3:6

French GL (2006) Bactericidal agents in the treatment of MRSA infections—the potential role of daptomycin. J Antimicrob Chemother 58:1107–1117

Gupta LK, Jindal R, Beri HK, Chhibber S (1992) Virulence of silver-resistant mutant of *Klebsiella pneumoniae* in burn wound model. Folia Microbiol (Praha) 37:245–248

Hoyme UB (1993) Clinical significance of Crede's prophylaxis in Germany at present. Infect Dis Obstet Gynecol 1:32–36

Ip M, Lui SL, Poon VK, Lung I, Burd A (2006) Antimicrobial activities of silver dressings: an in vitro comparison. J Med Microbiol 55:59–63

Kong H, Jang J (2008) Antibacterial properties of novel poly (methyl methacrylate) nanofiber containing silver nanoparticles. Langmuir 24:2051–2056

Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, Tam PK, Chiu JF, Che CM (2006) Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res 5:916–924

Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, Tam PK, Chiu JF, Che CM (2007) Silver nanoparticles: partial oxidation and antibacterial activities. J Biol Inorg Chem 12:527–534

Lut L, Sun RW, Chen R, Hui CK, Ho CM, Luk JM, Lau GK, Che CM (2008) Silver nanoparticles inhibit hepatitis B virus replication. Antivir Ther 13:253–262

Matsumura Y, Yoshikata K, Kunisaki S, 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 GE (2007) Chemical disinfection. In: Antisepsis, disinfection, and sterilization, pp 111–115

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Tapia J, Yacaman MJ (2005) The bactericidal effect of silver nanoparticles. Nanotechnology 16:2346–2353

Pal S, Tak YK, Song JM (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 73:1712–1720

Petica A, Gavriliu S, Lungu M, Buruntea N, Panzaru C (2008) Colloidal silver solutions with antimicrobial properties. Mater Sci Eng 152:22–27

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

Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D (2007) Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology 18:1–9

Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. J Colloid Interface Sci 275:177–182

Webb GF, D'Agata EM, Magal P, Ruan S (2005) A model of antibiotic-resistant bacterial epidemics in hospitals. Proc Natl Acad Sci USA 102:13343–13348

World Health Organization (2007) WHO Model of essential medicines. Online source

Yamanaka M, Hara K, 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