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REVIEW ARTICLE

Silver as an antimicrobial: Facts and gaps in knowledge

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Abstract

Silver has been used for centuries. Today, silver and silver nanoparticles (AgNPs) are used in a wide range of healthcare, food industry, domiciliary applications, and are commonly found in hard surface materials and textiles. Such an extensive use raises questions about its safety, environmental toxicity and the risks associated with microbial resistance and cross-resistance. If the mechanisms of antimicrobial action of ionic silver (Ag^+) have been studied, there is little understanding of AgNPs interactions with microorganisms. There have been excellent reviews on the bacterial resistance mechanisms to silver, but there is a paucity of information on resistance to AgNPs. Silver toxicity and accumulation in the environment has been studied and there is a better understanding of silver concentration and species in different environmental compartments. However, owing to the increased applications of silver and AgNPs, questions remain about the presence and consequences of AgNPs in the environment. This review provides an historical perspective of silver usage, an overview of applications, and combined information of microbial resistance and toxicity. Owing the evidence provided in this review, a call for a better understanding and control of silver usage, and for tighter regulations of silver and AgNPs usage is proposed.

Keywords: ionic silver, nanosilver, resistance, toxicity, activity

Brief historic and medical use

The use of silver can be traced back in history. Silver was used in ancient time to preserve water (use of silver vessels, use of silver coins) (Silver et al. 2006). Its use for medicinal purposes has been first documented in 750AD, although it may have occurred before that. In the 17th century, silver was described as an essential multipurpose medicinal product and was used to treat epilepsy and cholera (Edwards-Jones 2009). The first scientific paper describing the medical use of silver has been attributed to F. Crédé in the late nineteen century who used one-percent silver nitrate solution as eye drops in newborns, eliminating blindness caused by postpartum eye infections, and in 1901 for internal antisepsis (Russell & Hugo 1994). At the beginning of the 20th century the use of silver foil dressing was introduced by W.D. Halstead a surgeon; the dressing was listed on the Physician's Desk Reference until 1955 (Silver et al. 2006). The U.S. Food and Drug Administration (FDA) approved charged silver solutions (i.e. electrocolloidals) in the 1920s for use as antibacterial agents. At the same period, A.C. Barnes in Philadelphia invented Argyrol as a local antiseptic to prevent eye infections in particular. He recognized that silver nitrate eye-drops often were caustic to human tissues and that a more benign and effective silver product could be produced by absorption of Ag^+ on the surface of colloidal proteins such as gelatin.

The use of silver nitrate (0.5%) in compresses for the treatment of burn wounds was first explored by Moyer and colleagues (1965). Such application worked well at the time to control *Pseudomonas aeruginosa* infection, but the development of bacterial resistance to silver nitrate (Cason et al. 1966; Cason & Lowbury 1968) prompted a change in formulation and the use of silver sulphadiazine – a combination of silver and sulphonamide (Fox 1968; Modak & Fox 1974; Modak et al. 1988). It was proposed that such a combination functions with the slow release of Ag^+ as the primary microbicide while sulfadiazine serves mostly to keep Ag^+ in solution and to prevent the light-sensitive formation of black

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colloidal Ag^0 on the skin surface, again a serious cosmetic problem with $AgNO_3$-based products, since patients object to skin blackening (Klasen 2000). However, it has been suggested that bacterial resistance to silver sulphadiazine developed rapidly mainly because of the antibiotic component (Klasen 2000). Other combinations have then been explored such as combination of silver sulphadiazine with chlorhexidine (Fraser et al. 2004), silver sulphadiazine and cerium nitrate (Flammacerium) (Garner & Heppell 2005a, 2005b). Today the *British National Formulary* (2011) authorizes the use of silver nitrate (40–95%) for external use on warts, verruca, umbilical granulomas, over-granulating tissue, cauterization and silver sulphadiazine (1%) for the "prophylaxis and treatment of burn wounds, as an adjunct to short-term treatment of infection in leg ulcers and pressure sores, adjunct to prophylaxis of infection in skin graft donor sites and extensive abrasions" (BNF 2011). Other medical preparations are listed in Table 1.

During the second half of the 20th century silver was also used as a disinfectant especially in conjunction with hydrogen peroxide. The efficacy of numerous commercial products for the food industry, private swimming pools, surface and equipment disinfection were based on claims evidencing a synergistic effect. In fact it has been demonstrated (Hartemann et al. 1995) that silver acts as a catalyst in the Fenton reaction for producing free hydroxyl radicals.

The use of silver for healthcare applications has been briefly reviewed by Edwards-Jones (2009) who pointed out that its use should be justified financially by an evidence of cost benefit as illustrated by the use of nanocrystalline silver dressing or the potential use of silver-coated urinary catheters (Silver et al. 2006; Hsu et al. 2010; Apirag et al. 2011). It should be noted that the use of silver in catheters is still a matter of debate (Leone et al. 2004). Silver and especially nanosilver/silver nanoparticles (AgNPs) is now used in a number of dressings and evidence for activity has been reviewed by Silver et al. (2006) and more recently by Toy and Macera (2011). Silver has also been heavily used in dentistry in silver amalgams (Silver 2003)

Table 1. List of preparations using silver according to the Martindale (2002).

Silver acetate, silver borate, silver allantoinate, silver zinc allantoinate, silver carbonate, silver chloride, silver chromate, silver glycerolate, colloidal silver iodide, silver lactate, silver manganite, silver nylon polymers	Antiseptic (similar use to silver nitrate)
Silver nitrate (1%)	Prophylaxis of gonococcalophthalmianeonatorum (neonatal conjunctivitis)
Silver protein	Antisepsis; eye drops and mucous membrane
Colloidal silver	
Silver sulphadiazine (1%)	Prevention and treatment of infection in severe burns
	Eye treatment of *Aspergillus* infections

and silver containing products are used in medicine in an expanding range of applications. The most important current use is undoubtedly as a microbicide to prevent infections associated with long-term and recurrent sites including burns, traumatic wounds and diabetic ulcers. Additional uses include coating of catheters and other devices implanted on or within the body. Non-medical uses include home consumer products and disinfection of water and equipment, which expand tremendously the range of products containing silver and AgNPs.

The renewed interest in silver can be attributed to its bactericidal efficacy at a low concentration, relative limited toxicity of ionic silver to human cells, and recent advances in the production of nanoparticles and impregnation techniques and polymer technologies (Maillard & Denyer 2006a; Marambio-Jones & Hoek 2010). The combination of ionic silver and other forms of silver with other molecules and especially with polymers in dressings aim to increase the overall antimicrobial effect of silver and its sustainability, to decrease silver toxicity and interference with the dressing, and to improve wound healing and fluid handling (Maillard & Denyer 2006a, 2006b). There are now a number of silver-based dressings on the market and they differ widely in their structure, formulation and silver concentration (Kostenko et al. 2010; Toy & Macera 2011).

Silver applications

There has been a tremendous increase in applications using silver and AgNPs (Silver et al. 2006; Gottschalk et al. 2010) (Table 2). AgNPs might be the most commonly used nanomaterials in consumer products (300/1317 listed products contained AgNPs) (Project on Emerging Nanotechonologies 2011). AgNPs are usually defined as particles with at least one dimension measuring less that 100 nm. The interest in AgNPs rests with an improved microbicidal activity (Marambio-Jones & Hoek 2010), a perceived reduced overall toxicity and an ease of incorporation in a number of polymer or biomaterials. Some of the common attributes of AgNPs rest on a greater surface area, an improved bioavailability and greater chemicals and biological reactivity. At least two main methods are used to provide antimicrobial properties to polymers: (i) the introduction of silver nanoparticles at define concentrations into ready-made items and (ii) the introduction of silver nanoparticles into raw materials for subsequent manufacture of polymeric items. The methods to incorporate silver particle into polymers and other materials are often proprietary and are the source of the technology intellectual property.

The use of silver (50 ppm) in personal care products has been explored as early as 1996 with great success in terms of controlling skin flora without associated toxicity or hypersensitivity (Corbett 1996). The use of AgNPs is more recent with demonstration of microbial flora control for 24 h (Nakane et al. 2006).

Silver nanoparticles have been used in a wide range of matrices and formulations such as composites, colloids,

Table 2. Examples of silver and AgNPs applications.

Healthcare	Wounds dressings, antiseptics, hospital beds and furniture
Home consumer products	Fabric conditioners, baby bottles, food storage containers and salad bowls, kitchen cutting boards, bed mattress, vacuum cleaner, disposable curtains and blinds, tableware, independent Living Aids – bathroom products, furniture (chairs), kitchen gadgets and bath accessories, dishwashers, refrigerators and washing machines, toilet tank levers to sink stoppers, toilet seat, pillows, and mattresses, food storers, containers, ice trays, and other plastic kitchenware, hair brush, hair straightener, combs, brushes, rollers, shower caps
	Toothpaste, cosmetic deodorants, toothbrushes, tissue paper, epilator, electric shaver
	Pet shampoos, feeders and waters, litter pans, pet bedding and shelter, paper, pens and pencils, ATM buttons, remote control, handrails (buses), computer keyboards, hand dryers, wireless voice communicators with badge and the sleeves, yoga mat, coatings for use on laptop computers, calculators, sheet protectors, name badges and holders, shop ticket holders, media storage products, laminating film, report covers and project folders, photo holders, memory Book, office accessories, transparency film, collapsible coolers
Clothing and fabrics	Baby clothes, underwear, socks, footwear, various fabrics and vinyls, bath towels, quilts, sleeping bags, bed linens, pillows, quilts, mattress protectors and towels
Food	Packaging, nanobiotic poultry production
Construction	Powder coating (door knobs), wall paints, air conditioning, epoxy resin floor, PVC wall cladding, antimicrobial flooring, metal suspended ceiling systems, window blinds and shading systems, shelving systems, decorative wood laminates, electrical wiring accessories, notile panels (alternative to standard tiling), hygienic laminated surfaces, wallpaper, borders and murals, carpet and carpet underlay, seals (door for cooler doors and freezer cells, tank lids, mixers and kneading machines, hospital doors, for vibrating screens /vibrosieves in the pharmaceutical industry)
Disinfectants	Agricultural disinfectants, industrial disinfectants, aquaculture disinfectants, pool disinfectants

fibers, gels, coatings, membranes and thin films (Porel et al. 2011). Silver is also used in food packaging. For example, the efficacy of silver (-zeolite) to prevent *Pseudomonas putida* at low temperature was observed to be marginal over 2 days when compared from a silver-free packaging, but significant (>3 log) over 4 days (Lee et al. 2011). The use of AgNPs for liquid packaging has also been reported, with some very modest effect (1 log reduction of *Lactobacillus plantarum*) in 112 days (Emamifar et al. 2011).

Mechanisms of action of ionic silver

Efficacy of silver as an antimicrobial

Silver has a broad spectrum of antimicrobial activity against planktonic and sessile bacteria (Edwards-Jones 2009; Percival et al. 2011), although bacterial spores, protozoal cysts and mycobacteria are less affected. The activity of silver resides in ionic silver at a concentration of 10^{-9} to 10^{-6} mol/L, while Ag^0 is inactive. Silver ions activity can be enhanced with combination with other antimicrobial agents, antibiotics (e.g. sulphonamide; silver sulphadiazine), chlorhexidine (Silvazine™), cerium nitrate (Flammacerium). The bactericidal activity of AgNPs has been reported (Su et al. 2011) including against bacterial biofilms (Kostenko et al. 2010; Huang et al. 2011). AgNPs is thought to have a better activity than ionic silver (Rhim et al. 2006; Fernández et al. 2010a, 2010b; Marambio-Jones & Hoek 2010). AgNPs are thought to have the ability to generate more ionic silver, to increase the production of reactive oxygen species, notably when combined to halides, and to deliver more efficiently ionic silver to small surfaces (Wijnhoven et al. 2009). The bactericidal activity of AgNPs can be improved with combination with polymers such as chitosan and cationic polysaccharide (Banerjee et al. 2010). The production of free radicals in combination with H_2O_2 is also another way to enhance the "cidal" activity for disinfecting inanimate surfaces or water (Hartemann et al. 1995).

The activity of silver depends upon its bioavailability and the type of target microorganisms. It has been recognized that the activity of silver used for the treatment of burn wounds is limited and silver is unlikely to eliminate bacteria already colonizing the wound because of the lack of penetration of ionic silver and its rapid neutralization with organic matter. It has been estimated that the maximum concentration of available ionic sliver attainable in wound is 1 μg/mL (Maillard & Denyer 2006b). The bioavailability of silver is affected by silver concentration, although its concentration exponent is low, its poor solubility in water, its rapid adsorption to surfaces, its rapid precipitation when combined with chloride, sulphite and phosphate and its rapid neutralization by organic matter (mainly protein). In particular, the concentration of halides has been shown to have an effect on silver bioavailability, since high halide concentrations bring silver back into solution, improving antimicrobial activity against silver-susceptible and -resistant bacteria (Gupta et al. 1999a). In silver-impregnated dressing, the release of ionic silver is linked to the level of hydration (Lansdown et al. 2005). The amount of ionic silver release, the rate of release and long-term release are also parameters that may play an important role in the efficacy of silver dressings (Kostenko et al. 2010). The release of ionic silver also depends upon the nature of the silver antimicrobial and the polymer matrix used (Monteiro et al. 2009).

Silver activity is modestly affected by temperature or a change in pH (notably alkaline pH) (Maillard & Denyer 2006b). Duration of exposure is also an important parameter to consider for long-term usage devices. The use of silver in endotracheal tube was shown to prevent biofilm formation for a few days (Berra et al. 2008; Roe et al. 2008) but not in longer use (Olson et al. 2002).

Water hardness and notably the presence of divalent cations affect the bactericidal efficacy of AgNPs against Gram-positive in a liquid environment possibly through an increase size of nanoparticle aggregates and possibly a reduced binding to the bacterial surface (Jin et al. 2010). However, the presence of divalent cations seem to increase the efficacy of AgNPs against Gram-negative bacteria, possibly by increasing the interaction and 'local concentration' of the negatively charged silver-nanoparticle and the negatively charge lipopolysaccharide layer (Jin et al. 2010). The size and morphology of nanoparticles affect microbicidal efficacy (Lok et al. 2007; Pal et al. 2007; Samberg et al. 2011).

Mechanisms of action of silver

It has been proposed that ionic silver while inside the cell, interact with multiple target sites (Russell & Hugo 1994). Its antimicrobial activity results from its combination with, and alteration of, microbial proteins, with eventually structural and metabolic disruption (Maillard & Denyer 2006a; Silver et al. 2006). It has been suggested that once sufficient ionic silver has penetrated within the bacterial cell, recovery is improbable. It can be noted that the presence of moisture is required for the penetration of ionic silver within the cell. At the cell membrane level, ionic silver has been observed to inhibit the proton motive force, the respiratory electron transport chain, and to affect membrane permeability resulting in cell death (Percival et al. 2005; Silver et al. 2006; Edwards-Jones 2009). The virucidal effect of ionic silver is thought to occur through an interaction with viral proteins and or viral nucleic acid (Maillard 2001).

A number of studies have proposed both intracellular and extracellular explanations to explain the activity of AgNPs (Morones et al. 2005; Gogoi et al. 2006; Lok et al. 2006; Banerjee et al. 2010). Silver nanoparticles up to 80 nm have been shown to be able to penetrate the inner and outer bacterial cell membrane (Xu et al. 2004). AgNPs of less than 10 nm diameter were observed to cause cytoplasmic leakage by forming pores on the bacterial cell wall without affecting extracellular proteins and bacterial nucleic acid (Gogoi et al. 2006). The release of ionic silver from AgNPs (when it occurs) does not appear to be responsible for the observed "cidal" activity of AgNPs (Lok et al. 2006; Su et al. 2009; Miyoshi et al. 2010). Su et al. (2009) demonstrated that bactericidal effect of silver nanoparticles immobilized on a surface was caused by the loss of membrane integrity due to reactive oxygen species, while the energy-dependent metabolism was inhibited. A combination of nanosilver and iodine as shown to damage bacterial cell wall and produced reactive oxygen species causing oxidation damage in the cell cytoplasm leading to cell death (Banerjee et al. 2010).

Bacterial resistance to ionic silver

Occurrence of resistance to silver

Silver resistance in bacteria following the use of silver has been well documented. Probably the first report of documented resistance in practice follows the use of silver nitrate and silver sulphadiazine for burn wound treatment. Cason et al. (1966) first reported silver resistance in *Pseudomonas aeruginosa* associated with burn wounds. A number of reports highlighting outbreaks of burn wound infection or colonization by Gram-negative isolates resistant to ionic silver and silver sulphadiazine have since emerged; in *Enterobacter cloacae* (Gayle et al. 1978), *Providencia stuartii* (Wenzel et al. 1976), *Pseudomonas aeruginosa* (Bridges et al. 1979), *Salmonella* Typhimurium (Mchugh et al. 1975) emerged soon after. Silver-resistance from environmental bacterial isolates has since been well documented in *Enterobacteriaceae* (Hendry & Stewart 1979; Kaur & Vadehra 1986; Starodub & Trevors 1989, 1990) and in *Acinetobacter baumanii* (Deshpande & Chopade 1994).

It has been suggested that exposure to silver might contribute to the selection of bacteria that are intrinsically resistant to silver (Wenzel et al. 1976; Bridges & Lowbury 1977; Haefeli et al. 1984; Silver 2003; Davis et al. 2005).

In the laboratory, high-resistance to silver (>1024 ppm) in *Escherichia coli* has been produced following step-wise training (i.e. repeated exposure to increasing concentration) (Li et al. 1997).

It is interesting that the available reports on the development of bacterial resistance to silver concern Gram-negative bacteria. There is a lack of evidence in emerging silver resistance in Gram-positive bacteria, possibly because Gram-positive bacteria are, at least for some genera, less susceptible to silver (Cason et al. 1966; Spacciapoli et al. 2001).

Mechanisms of resistance to silver

Since it appears that the lethal effect of ionic silver follows its penetration into the cell cytoplasm, the main mechanisms conferring silver resistance involve reducing ionic silver penetration via a non-specific transporter (Nies 1999), reducing accumulation (i.e. increasing in silver efflux) (Silver 2003) and reducing its concentration by increased neutralization and reduction of Ag^+ to the inactive metallic form (Nies 1999).

For example, in a silver-resistant *Escherichia coli* produced by stepwise training, active efflux and outer membrane protein changes accounted for the high resistance of the strain to ionic silver (Li et al. 1997). Kaur and Vadehra (1986) observed a similar silver uptake between a *Klebsiella* strain resistant to silver (70 μg/mL) compared to a silver-sensitive parent strain (10 μg/mL). Since the ionic silver uptake of spheroplasts of both strains was also similar, the difference in susceptibility was attributed to a change in cell membrane composition. The activity of succinate dehydrogenase was also reduced in the silver-resistant strain (Kaur & Vadehra 1986). However, Starodub and Trevors (1989, 1990) observed differences in silver binding to and accumulation in *Escherichia coli*, between a silver-resistant isolates (1 mM silver nitrate) and a silver-sensitive construct derived from the isolate

cured of its plasmid conferring silver resistance. The formation of an inactive, insoluble silver sulphide following the chelation of silver by the sulfhydryl groups of metal-binding proteins has also been described (Liau et al. 1997), while exopolysaccharide could be involved in reducing the concentration of ionic silver (Miao et al. 2009).

Bacterial resistance to AgNPs has been described (Lok et al. 2007; Samberg et al. 2011; Hsu et al.2010). The exact mechanisms conferring resistance to AgNPs are likely to differ in part from mechanisms conferring resistance to ionic silver since AgNPs has been shown to be effective against silver-resistant bacteria. Overexpression of detoxification enzymes and membrane repair related proteins have been suggested as mechanisms involved in AgNPs resistance (Simon-Deckers et al. 2009). A reduction of AgNPs penetration or accumulation might be explained partly by the interaction of AgNPs with the outer membrane of Gram-negative bacteria. The presence of lipopolysaccharide might induce electrostatic repulsion with negatively charged silver nanoparticles (Costerton et al. 1974) while phosphomonoester function group and carboxyl groups could complex with ionic silver (Guiné et al. 2006).

Transfer of resistance

Silver resistance in bacteria has been found to be often encoded on plasmid (McHugh et al. 1975; Gupta et al. 2001; Davis et al. 2005) and has been described in a number of Gram-negative bacteria such as *P. aeruginosa, Pseudomonas stutzeri*, *Citrobacter* spp., *Serratia marcescens* and *Salmonella enterica* serovar Typhimurium has been documented (Silver et al. 2006). It has also on occasions been described on bacterial chromosome (Silver & Phung 1996, 2005; Gupta et al. 2001).

In silver-resistant *S. enterica* Typhimurium isolated from a burns unit, silver resistance was encoded on the plasmid pMG101, also conferring multi-drug resistance (Silver 2003). The plasmid contained *silCBA* that encodes a resistance nodulation division (RND) efflux pump with homologues to that of AcrB in *E. coli* (Silver 2003), *silE* encoding for periplasmic silver-binding protein, SilE which bind ionic silver (Silver et al. 2006). SilA is an inner membrane cation pump protein while SilC an outer membrane protein (Silver et al. 2006). The silver resistance determinant has been described as unique among resistance system since it encodes for two energetically distinct efflux pump (Figure 1) (Silver et al. 2006). The *sil* genes have been found to occur only on IncH incompability group plasmids (Silver et al. 2006). The occurrence of silver encoded plasmid in enteric bacterial isolates from a hospital was found to exceed 10% (Silver 2003). In *A. baumanii* silver-resistance encoded on a 54 kb plasmid was transferred successfully to *E. coli* by conjugation. The transformed *E. coli* cells were shown to be more efficient to efflux accumulated silver ions (Deshpande & Chopade 1994).

Loh et al. (2009) reported that the presence of silver-resistance genes in methicillin-resistant *Staphylococcus aureus* (MRSA; 33 isolates) and methicillin-resistant coagulase-negative *S aureus* (MR-CNS; 8 isolates) isolated from wounds and nasal cavities in human and animals was low (2 /33 MRSA and 1/8 MR-CNS) and restricted to a single gene (*silE*). In addition isolates with the *silE* genes remain susceptible to a silver-containing hydrofiber wound dressing (Loh et al. 2009). Another study investigating the presence of silver-resistance genes in 172 isolates from human (112) and equine chronic wounds (60) reported that only 6 isolates, all *Enterobacter cloacae* (2 from human and 4 from horses), possessed the resistant *sil* gene cassette (Woods et al. 2009). All the silver-resistant genes were present extrachromosomally. The *sil* gene cassette in these isolates conferred a resistance of > 5 mg/L MIC, compared to a 1.25 mg/L in the *sil*-negative strains. It was further reported that a silver-containing dressing killed the *sil*-positive and -negative strains within 30 min, although the *sil*-positive strains were overall more resilient to the silver dressing (Woods et al. 2009).

Plasmid encoded silver resistance is of a particular concern since plasmid mediated metallic salt resistance is associated with co-resistance to chemotherapeutic antibiotics (Mchugh et al. 1975; Gupta et al. 1999b) and that silver resistance might persist in the clinical setting (Gupta et al. 2001). This is particularly pertinent following the widespread use of silver in hospital products, devices and environmental surfaces.

Toxicity of silver

Cell and human toxicity

The first well-known and well-described complication of silver ingestion or application in human is argyria. Argyria occurs when subdermal Ag deposits results in an irreversible gray to blue-black coloring of the skin. It is the rare result of ingesting large amounts of silver preparations usually as health stimulants (Russel & Hugo 1994). Argyria is permanent but not physically harmful; it is however an inherent serious cosmetic problem. The most familiar human exposure to Ag is from dental amalgams that contain 35% Ag (0) and 50% Hg (0) (Dunne et al. 1997).

The use of ionic silver and silver derivatives for treatment and prevention of infection of burn wounds or skin grafting has been associated with a number of side effects such cytotoxicity, staining, methaemoglobinaemia and electrolyte disturbance, longer slough separation time, retardation of wound healing and the possible inactivation of enzyme debriding agents. The development of new dressings that allow the slow but sustained release of ionic silver while enhancing wound healing and fluid handling is contributing to a decrease in side effect together with an increase in antimicrobial activity (Maillard & Denyer 2006b).

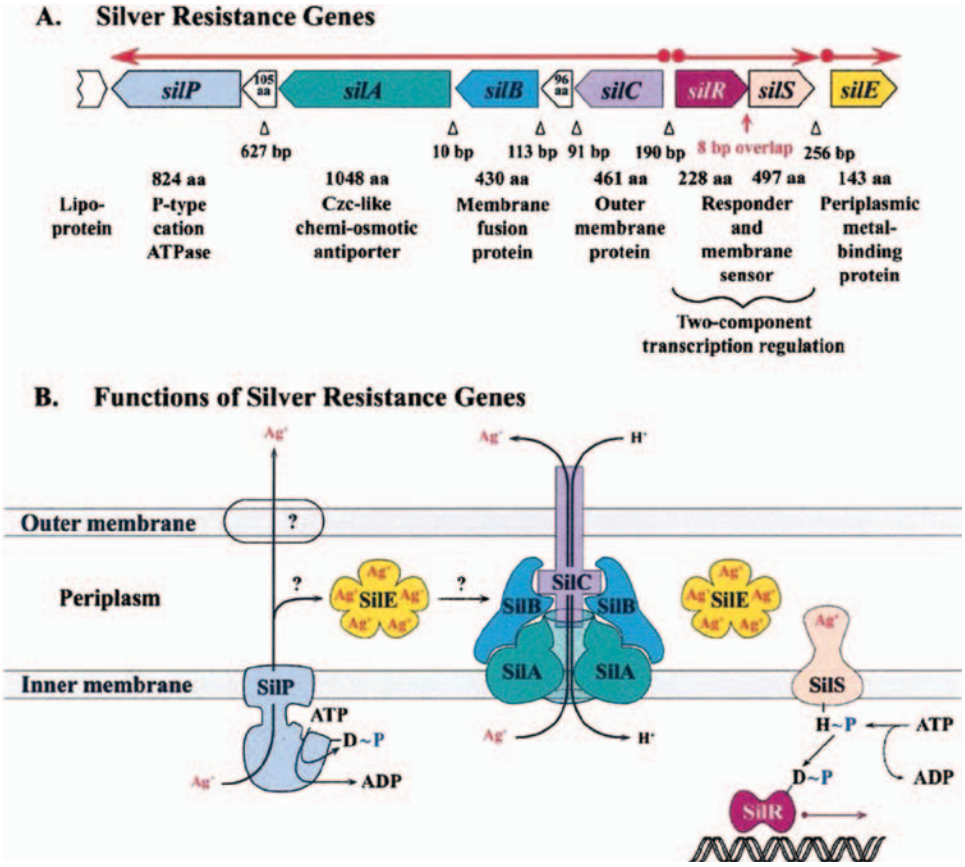

Figure 1. Protein products of bacterial plasmid silver resistance genes (from Silver 2003). Reproduced with permission of FEMS Microbiology Reviews. (See colour version of this figure online at www.informahealthcare.com/mby)

Investigations on the toxicity of metal particulates, including AgNPs, are still in their infancy at this time and have concentrated on revealing the toxicity and tissue distribution of silver. Consequently, more comprehensive studies are required to fully understand the toxicity associated with metal particulate exposure. AgNPs might present a potential hazard to human health since they can become systemically available following exposure as evidenced by their preferential accumulation within the liver. Silver accumulates within the liver and this may be associated with toxicity and inflammatory responses. However, the contribution of the liver to the elimination of silver notably through the bile and the reticuloendothelial system needs further investigating. The appearance of argyria, which is reliant on the accumulation of silver within the skin, has transpired following silver ingestion, further emphasizing the propensity of silver to become systemically available (Johnston et al. 2010). According to these authors it is apparent that the toxicity of silver particulates depends on their internalization and oxidative nature, which drives inflammatory, genotoxicity, and cytotoxic events. The toxicity of AgNPs might be better controlled by the prevention of ionic silver leaching and a better design of the AgNPs impeding penetration into mammalian cells, thus avoiding deposition of silver in the body (Su et al. 2011).

Concentrations of AgNPs (5 μg/mL and 10 μg/mL) have been shown to induce necrosis and apoptosis of mouse spermatogonial stem cells (Braydich-Stolle et al. 2005). The toxicity of AgNPs might be mediated by the concentration of ionic silver released (Miao et al. 2009), although complexation with cysteine (a strong ionic silver ligand) may enhance overall toxicity (Navarro et al. 2008). Kim et al. (2009) suggested that oxidative-stress might be a mechanism involved in the cell toxicity of AgNPs. The size and morphology of nanoparticles affect toxicity (Liu et al. 2010).

Environmental toxicity

Accumulation of heavy metal in the environment has been mentioned by the Agency for Toxic Substances and Disease Registry (ATSDR 2012) and the European Commission (1998). Silver may also accumulate in the environment although to a lesser extent than other heavy metals.

There is a general agreement that dissolved silver ions are responsible for the biological toxic action that is especially pronounced against microorganisms (Navarro et al. 2008; Choi et al. 2008; Hwang et al. 2008). Silver is present in the environment primarily as silver sulfides and silver chloride ($AgCl^{1}_{n}{}^{-n}$) complexes (Cowan et al. 1985; Kramer 1995). Dissolved organic carbon (DOC) and colloid complexes with silver are probably also prevalent (Sujari & Bowen 1986; Wen et al. 1997). While silver sulfide dominates under reducing conditions, the other silver

species are more important in oxidizing waters where fish are likely to reside. The free silver ion (Ag+), which is undoubtedly the most toxic species of silver, is present at concentrations worth consideration only in freshwater, not in brackish water or seawater (Hoogstrand & Wood 1998). The existence of various silver species depends on physicochemical environmental conditions.

The silver ion is one of the most toxic forms of a heavy metal, surpassed only by mercury and thus has been assigned to the highest toxicity class, together with cadmium, chromium (VI), copper, and mercury (Doudoroff & Katz 1953). Annual silver released to the environment from industrial wastes and emissions has been estimated at approx. 2,500 tonnes, of which 150 tonnes gets into the sludge of wastewater treatment plants and 80 tonnes is released into surface waters (Smith & Carson 1977; Petering 1984).

Obviously, the perception of high silver toxicity has long been due to the fact that most laboratory toxicity experiments tested $AgNO_3$, which readily dissolves, releasing the highly toxic free Ag^+ ion (Ratte 1999). Because of enhanced heavy metal analyses and experimental techniques (e.g. the ultraclean technique) (Benoit et al. 1994; Cutter & Radford-Knoery 1994; Willie et al. 1994; Shafer et al. 1998; Schildkraut et al. 1998), a better understanding of total silver concentrations in various environmental compartments and, in particular, of silver speciation has emerged.

Silver sulfide, silver thiosulfate complex and silver chloride complexes were tested and compared with free silver ion for their toxic against fathead minnow (*Pimephales promelas*) (Le blanc et al. 1984). Silver chloride complexes, silver sulphide and silver thiosulfate complex were about 300 times, at least 15,000 times, and more than 17,500 times less acutely toxic, respectively, than free silver ion. The estimated maximum acceptable toxicant concentrations (MATCs) determined from the embryo larval tests with silver sulfide thiosulfate complex were greater than 11 mg/L (as total silver). The MATC previously reported for free silver ion from tests with rainbow trout (*Salmo gairdneri*) was greater than 0.00009 but less than 0.00017 mg/L. These differences in acute toxicities and the differences of five orders of magnitude in the MATC values are attributed to the influence of chemical speciation on the effects of silver in the aquatic environment. Thus, the speciation of silver is an essential factor in its potential to affect fish and, presumably, other aquatic life, and therefore should be considered in environmental risk assessment.

Research evidence demonstrates that apparent toxicity is related to individual silver species rather than total silver concentration. In natural waters, where silver contamination can be of concern, evidence of toxicity from the dissolved silver ion is generally less than in laboratory tests because of the rich opportunities for possible covalent, complexing, or colloidal hinding silver encounters with a variety of reactant (Adams & Kramer 1998; Shafer et al. 1998; Schildkraut et al. 1998).

The majority (>94%) (Shafer et al. 1998) of the silver released into the environment will remain in the soil or wastewater sludge at the emission site. A portion, the toxicity of which should not be underestimated, will be transported for long distances by air. Silver from industrial and public wastewater is bound to the activated sludge of wastewater treatment plants. The remaining portion of the silver enters the aquatic environment and, under freshwater conditions, it will be immediately adsorbed to sediments or suspended particles. Silver is kept in solution by colloidal and complex material will be transported downstream and enter lakes, estuaries or the sea. However, there is no evidence of substantial biomagnifications of silver in aquatic organisms to date (Bard et al. 1976; Terhaar et al. 1977; Biddinger & Gloss 1984; Forsythe et al. 1996; Galvez et al. 1996).

Gaps in knowledge

Testing the efficacy of silver-containing products

There are many products that incorporate, or are impregnated or coated with silver or AgNPs (Table 2). The methodologies to test the antimicrobial efficacy of fabrics and surfaces are based on the principle of diffusion from the coated/impregnated surface through liquid or moisture by immersion of the material in a broth, or by placing the material onto a nutrient surface conducive for microorganisms and antimicrobials. Most environmental surfaces or textile would contain very little moisture and with this in mind, the current standard efficacy tests such as the ISO 22196 (2011) or JIS Z 2801 (2000) do not reflect the usage of the materials in conditions that reflect the practice and may provide an overestimation of their antimicrobial efficacy.

The parameters used for testing the antimicrobial efficacy of antimicrobial surfaces are paramount. For example, in a number of studies investigating the activity of silver dressings against bacterial biofilms, results from *in vitro* experiments (Percival et al. 2008; Shahverdi et al. 2007; Thomas & McCubbin 2003; Yin et al. 1999) differed dramatically the results obtained from *in vivo* studies (Heggers et al. 2005). However, experimental parameters do not explain all the differences in results between *in vitro* and *in vivo* studies. An *in vitro* study mimicking conditions found *in situ* observed differences in antimicrobial efficacy of a number of silver dressing against bacterial biofilm (Kostenko et al. 2010). Nevertheless, the use of a test reflective of the usage conditions of the antimicrobial products would be a huge improvement from the current situation.

Silver resistance

The distinction between silver-sensitive and silver-resistant bacteria needs to be better clarified notably in relation with experimental parameters such as concentrations of halides. The clinical implication of silver-resistant bacteria and the presence of silver-resistance genes in environmental isolates need to be established.

The presence of plasmid mediated silver resistance is of concern (Gupta & Silver 1998). Such transferable resistance has been associated with the emergence of multidrug resistant microorganisms, although the extent of such transfer, and the subsequent associated risks, has not been appropriately assessed in the healthcare environment.

The bulk of evidence of emerging silver-resistance in bacteria concerns Gram-negative species. We are not aware of publications on the development of silver–resistance in gram-positive. This could be a serious omission owing the importance of Gram-positive bacteria, notably staphylococci, in human diseases.

Overall the mechanisms involved in silver resistance and their occurrence have been little studied; the gap in knowledge is particularly apparent with resistance to AgNPs. This is particularly pertinent when one considers the number of products containing AgNPs.

Silver mechanisms of action and toxicity

It is clear that AgNPs applications will increase in the future as the demand for applications is there. There is a need for a better understanding of these parameters on the efficacy and toxicity of AgNPs because of the wide variety in size, concentration, shape, materials, polymer and surfaces used.

Further work is needed to understand better the interactions of AgNPs with different bacterial cell types and other microorganisms. Bactericidal effect is likely to be multifactorial and the effect of external factors such as the presence divalent cations may play an important role in the efficacy of silver. More evidence of the mechanisms of action of AgNPs attached on a surface, without the leaching of ionic silver is needed together with a better understanding of the generation of reactive oxidative species in the mechanisms of action of AgNPs.

The bioavailability of AgNPs in the environment is paramount to understand potential toxicity, unfortunately this information is lacking.

Further considerations

Silver has been used for its antimicrobial activity for centuries. Advances in polymeric sciences has provided a renewed interest of silver for some medical applications such as dressings for which tangible benefits such as increased activity and reduced side effect have been observed. The AgNPs market is a buoyant one and is expected to expand rapidly (Gottschalk et al. 2010). It has been estimated that the market size for AgNPs was 320 T/year in 2009, which far exceeds (×10000) the volume of bulk silver (Gottschalk et al. 2010).

The wide use of silver and AgNPs (at a low concentration) in other applications such as fabrics, textiles and other surfaces may appear controversial and will remain controversial as long as the benefits have not been addressed, measured and justified appropriately. A recently published report on AgNPs usage highlighted the concern that the over use of AgNPs might lead to emerging silver-resistance but also resistance to antibiotics. A panel of experts agrees that the use of AgNPs should be better regulated (Crocetti & Miller 2012).

While the benefits of silver are widely recognized, little attention has been paid to the potential risks of continuous use of silver and its possible contribution to microbicide and antibiotic resistance needs to be evaluated.

Very few countries have a framework in place to regulate the sale of treated articles. In the EU, the issues or articles with internal and external effect are very complicated. It has been agreed that if an article has an external effect, it should be regarded as a biocidal product, but precisely how to do this is not agreed. The preferred answer seems to be to regulate the active substance, or formulation used and to view the article as a delivery system.

The US EPA classified silver ions and colloidal silver as a microbicide. Colloidal silver is on the unapproved EFSA list of food supplement and as such it cannot longer be legally sold as food supplement in the EU. Manufacturers have thus relabeled their colloidal silver-based product as "water disinfectants."

Declaration of interest

The authors report no conflicts of interest.

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