MOLECULAR GENETICS

Silver as a biocide: Will resistance become a problem?

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Human encounters with silver-containing products are surprisingly numerous worldwide[1-3], primarily as a biocide or antimicrobial agent. In water usage, silver- and copper-based disinfectants are used in hospital and hotel distribution systems to control infectious agents (for example, *Legionella*). Silver, together with copper, is commonly used to inhibit bacterial and fungal growth in chicken farms and in postharvest cleaning of oysters. Silver is used to sterilize recycled water aboard the MIR space station and on the NASA space shuttle[4]. Popular home water purification units in the United States contain silverized activated carbon filters along with ion-exchange resins. In Mexico, Microdyn (colloidal silver in gelatin) is sold in supermarkets to disinfect salad vegetables and drinking water. A quick surf on the Internet shows that silver is offered by several companies in different forms, and marketed as a health food additive—"Nature's alternative to antibiotics"—although the US Food and Drug Administration (FDA; Rockville, MD) has proposed that over-the-counter drug products containing colloidal silver or silver salts are not generally recognized as safe or effective for internal or external use and are frequently misbranded.[5]

Other silver-containing products continue to appear. Johnson Matthey Chemicals (Nottingham, UK)[6] has developed an inorganic composite (immobilized slow-release silver product) for use as a preservative in cosmetics, toiletries, and similar retail hygiene-sensitive products. In Japan, a new compound (Amenitop, silica gel microspheres containing a silver-thiosulfate complex) is mixed into plastics for lasting antibacterial protection. Metallic silver-copper containing ceramic disks are marketed as an alternative for users allergic to laundry detergents. Silver halide is often incorporated into prescription eye glasses for reversible "photochromatic" protection, as it decreases transmitted visible light. The US National Institute for Standards and Technology (Gaithersburg, MD) is developing a mercury-free tooth-filling material, still silver-based and with binding strength similar to the familiar amalgam.

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The medicinal uses of silver have been documented since 1000 B.C. Silver is a health additive in traditional Chinese and Indian Ayurvedic medicine[7]. And until recently, silver salts were administered to the eyes of newborn infants to prevent eye infections, a practice in Western medicine for nearly 200 years. Silver sulfadiazine (Silvadine, Hoechst Marion Roussel, Kansas City, MO, or Flamazine is a topical cream used on burn wounds, and metallic silver or silver-coated indwelling catheters are widely used to prevent infections.

Silver resistance is important to monitor because modern technology has developed a wide range of products that depend on silver as a key microcidal component.

Bacterial resistance to toxic agents is often found under conditions where the toxicity might select for resistance. Effluent from photography laboratories and silver sulfadiazine-treated burn patients are such environments. Nevertheless, skepticism remains as to whether silver-resistant bacteria actually occur and might cause environmental or clinical problems. Silver-resistant bacteria have been reported[8], and there have been incidences associated with invasive burn wound infections[9,10].

Until recently, a molecular genetic basis for bacterial resistance to silver was not known. Even the conditions that optimally differentiate between silver-sensitive and resistant growth were not clear. For example, halide levels in growth medium significantly alter the sensitivity/resistance pattern[1] and yet tissue on wounds is covered with plasma containing high levels of chloride and proteins, both of which bind silver ions.

We have recently cloned and sequenced the silver resistance determinant from a burn ward isolate of *Salmonella*[10] (A. Gupta, unpublished data). This plasmid system contains seven genes plus two less-recognized open reading frames. The gene products are homologous to proteins known for other metal resistance systems. Resistance to silver ions starts with SilE, a small periplasmic silver-binding protein that binds silver ions specifically at the cell surface, thus protecting the cell from toxicity. Products of the next two genes are a two component regulatory membrane kinase sensor (SilS) and transcriptional responder (SilR) that are thought to control mRNA transcription in silver resistance. The last four genes, *silCBAP*, are transcribed in the opposite direction, and their products appear to form two parallel efflux pumps that protect the cellular interior by pumping out silver cations that slip past SilE and enter the cell.

SilCBA is a three-protein, membrane potential–dependent, silver ion/hydrogen ion antiporter, and SilP belongs to the family of P-type efflux ATPases. Many heavy metal resistances result from efflux pumping of the metal ion from the cell and/or from chelation by metal-binding proteins (often using cysteines). Silver resistance includes both binding and efflux. However, contrary to expectation, there are no cysteines in the periplasmic protein SilE. The purified protein binds silver coordinately to the 10 histidine residues (J.-F. Lo and A. Gupta, unpublished data).

With the availability of the genes for silver resistance, we have identified closely related genes in bacteria from environmental and clinical environments and from diverse geographical locations (in preparation). This should eliminate skepticism about the existence of silver-resistant bacteria. The current widespread and uncontrolled use of silver may result in more bacteria developing resistance, analogous to the emergence of antibiotic- and biocide-resistant bacteria[11]. This could be very detrimental to the many industrial and medicinal products that depend on the microcidal properties of silver.

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