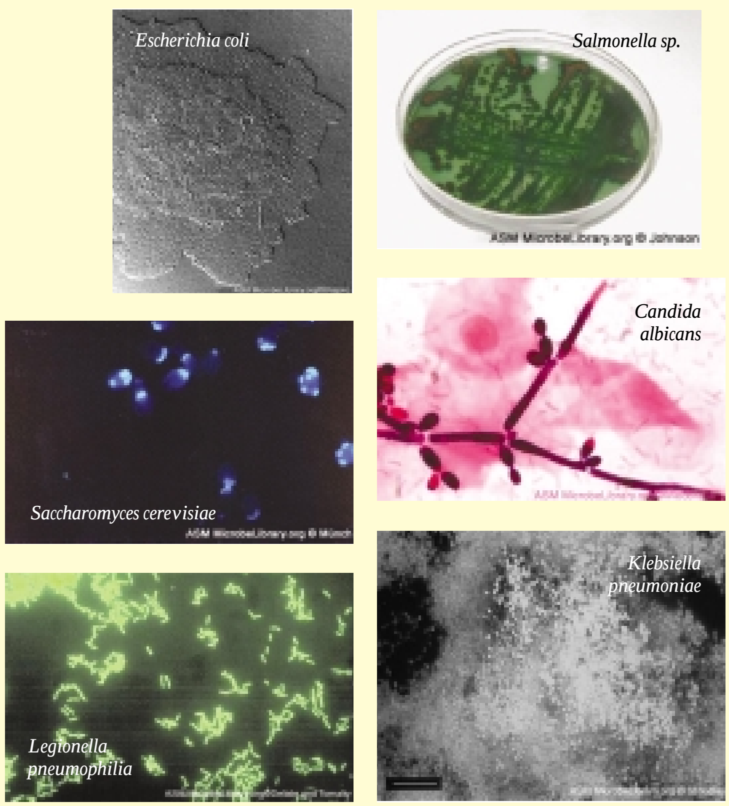
By W. Craig Meyer

Coping with Resistance to Copper/Silver Disinfection

The biocidal effects of copper and silver have been used for centuries. The early Greeks and Romans made water storage and drinking vessels out of these metals, and enough dissolved in the water stored in them to produce substantial disinfection. More recently, copper and silver ions have been used in hospital, recreational, drinking and industrial water systems. Unlike chlorine, they do not result in dangerous halogenated organic by-products such as trihalomethanes (THM), chloramines and chloroform, and these ions are stable, making it easier to maintain an effective residual. However, using soluble metal salts as a source of these ions and monitoring their concentrations to maintain consistent effects is cumbersome at best . Consequently, most modern copper/silver systems use electrolytic ion generators to control the concentrations of the dissolved metals.

Electrolytic generators usually are composed of a negatively charged cathode and a positively charged anode made of the metal or an alloy of the metals to be ionized. The electrodes are contained in a chamber through which passes the water to be disinfected. A DC power source provides current at a potential of a few volts, causing the copper and silver in the anode to ionize and dissolve in the passing water. The concentration of metal ions in water leaving the electrolytic cell depends on the current and water flow past the electrodes. Therefore, production of metal ions can be controlled by the current applied to the electrodes while the rate at which water flows through the chamber determines the concentration of dissolved ions.

Copper/Silver Resistant Microorganisms



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How Copper/Silver Works

The biocidal effect of copper and silver stems from a combination of mechanisms.[1,2] Positively charged silver and copper ions have an affinity for electrons and, when introduced into the interior of a bacterial cell, they interfere with electron transport in cellular respiration systems. Metal ions will bind to the sulfhydryl, amino and carboxyl groups of amino acids, thereby denaturing the proteins they compose. This renders enzymes and other proteins ineffective, compromising the biochemical process they control. Cell surface proteins necessary for transport of materials across cell membranes also are inactivated as they are denatured. Finally, copper will bind with the phosphate groups that are part of the structural backbone of DNA molecules. This results in unraveling of the double helix and consequent destruction of the molecule.

Resistance to Heavy Metals

The fact that copper/silver ions exert a microbiocidal effect cannot be argued. It is common to read in promotional brochures and hear in chat room discussions that copper/silver ions will accomplish any required biocidal task. However, a search of the literature reveals that many microorganisms (Table 1) including bacteria, protozoa, yeast, fungi and viruses are not effectively killed by exposure to these heavy metals.[3,4,5,6,7]

Frequently, in discussions with proponents of copper/silver systems, it has been stated that bacteria cannot develop resistance to copper/silver systems. However, bacteria are the most adaptive organisms known and have been shown to develop resistance to the spectrum of bactericidal agents including antibiotics and heavy metals. Many metal resistance mechanisms in bacteria are recognized.[8] Bacteria generate cell surface proteins that bind heavy metals, producing a barrier that prevents the metals from entering the cell. Other metal detoxification proteins are produced in the cytoplasm of bacteria and other organisms including yeast, fungi and even cells of multicellular invertebrates and vertebrates. These small (30 to 50 kd) cytoplasmic proteins are given a variety of names including metallothioneins, metal-binding proteins, cysteine-rich membrane-bound proteins, sequestering proteins and others. They all work because they bind to copper, silver and a host of other heavy metals. When bound to the amino acids (e.g., cysteine) on metallothionein-like detoxification proteins, copper and silver are isolated effectively from the other aspects of cellular chemistry and cannot exert their toxic effect. These proteins are simple products of single genes and are amplified easily to develop increased metal resistance. Bacteria also can exclude copper and silver that has reached the cell's interior. Efflux pumps (active biochemical) transport systems, bind to silver or copper and transport them to the cell surface where they are ejected. Finally, enzymes and other proteins that are the sites of toxic action often will become modified to reduce their sensitivity to copper and silver that may have escaped other detoxification mechanisms.

Plasmids (i.e., small gene-bearing rings of DNA) that encode resistance for Silver, Arsenic, Cadmium, Chromium, Copper, Mercury, Nickel, Lead, Antimony, Thallium and Zinc have been isolated from bacteria.[9] Bacteria exchange genetic material by conjugation, during which a tubular extension from the cell membrane of one bacterial cell is extended to connect with the membrane of another. Once the connection is established, genetic material is exchanged between the cells. This behavior is quite promiscuous, and bacteria of entirely different species and genera can exchange genes in this way. This means that genes for resistance developed by one species of bacteria can be rapidly spread to others. Considering their rapid reproduction rate and the ability to share genes between individuals of the same and different species, it is not surprising that resistance spreads very rapidly through the bacterial community.

Table 1: Some Microorganisms That Are Resistant to Copper and/or Silver

Organism	Type of Organism	Metal Resistance
Escherichia coli	bacteria	Cu
Klebsiella pneumoniae	bacteria	Cu & Ag
Legionella pneumophilia	bacteria	Cu & Ag
Salmonella sp.	bacteria	Ag
Vibrio cholerae	bacteria	Cu & Ag
Candida albicans	yeast	Cu
Saccharomyces cerevisiae	yeast	Cu & Ag
Hartmenella vermiformis	protozoa	Cu & Ag
Tetrahymena pyriformis	protozoa	Cu & Ag
Paramecium sp.	protozoa	Cu & Ag
Amoeba sp.	protozoa	Cu & Ag

Most studies of copper/silver disinfection report either single exposures to bacteria under laboratory conditions or field trials where a contaminated water system was equipped with a copper/silver system and results monitored for a season or less. In both cases, the initial effects were good, since the bacteria had not had sufficient time to develop or amplify resistance genes and pass them about through conjugation. If the results of these single exposure studies are assumed to be persistent, then confidence in the continued effectiveness of this disinfection technique is supported.

However, if these experiments were continued for several years, development of resistant strains would be expected. One such extended study of the effects of silver/copper disinfection on *Legionella* in a German hospital water system has been published.[10] In this case, a university hospital's hot water system contaminated with *Legionella* was fitted with silver/copper ionization to treat the problem. This system was monitored for four years to evaluate effectiveness. Initially, silver concentrations were not allowed to exceed 10 ppb and *Legionella* counts were reduced from 40,000 cfu/L to 7 cfu/L, a significant 3.8-log reduction. By the third year, *Legionella* counts had increased to 10,000 cfu/L. During the fourth year, silver concentrations were raised to 30 ppb, which produced only a 1.3-log reduction to 500 cfu/L. Based on the declining effectiveness of the original silver concentration and the poor response to tripling the concentration in the last year, the authors concluded, "*Legionella* developed a resistance to silver ions."

Recommendations

Numerous facilities have invested in copper/silver disinfection systems to address the limits of traditional water treatment methods. It seems likely that, as bacterial populations develop resistance, many of these systems will become less effective through time. In order to protect the investment in these technologies and provide effective management of disease-causing pathogens and the resulting legal exposures, the following recommendations should be considered.

First, look for a different disinfectant that will complement copper/silver systems and can be alternated with them on a regular basis. Secondly, considering that any population of bacteria would be expected to develop resistance after a period of exposure, it may be best to periodically eliminate the entire bacterial population. Where possible, water systems should be drained, cleaned and shocked with chlorine, ozone or another aggressive disinfectant. Even when this is done, it is probable that the promiscuous sharing of resistance genes between bacterial species and genera will result in eventual spreading of resistant bacteria to city water supplies. If so, this would result in the system being inoculated with resistant strains on refilling. Consequently, a regular drain and shock program is not a replacement for biocide alternation, and both these approaches should be utilized.

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References

1. Yahya, M.T. et al. "Disinfection of Bacteria in Water Systems by using Electrolytically Generated Copper: Silver and Reduced Levels of Free Chlorine." *Can. J. Microbiol.* Feb: 36(2):109–16, 1990.

2. Lin, Z. et al. "Controlled Evaluation of Copper-Silver Ionization in Eradicating Legionella pneumophilia from a Hospital Water Distribution System." *J. Infect. Dis.* Apr: 169(4):919–22, 1994.

3. Nies, D.H. "Microbial Heavy-Metal Resistance." *Appl. Microbiol. Biotechnol* Jun: 51(6):730–50, 1999.

4. Rohr, U. et al. "Impact of Silver and Copper on the Survival of Amoebae and Ciliated Protozoa in Vitro." *Int J Hyg Environ Health* Mar: 203(1):87–9, 2000.

5. Riggle, P.J. and C.A. Kumamoto. "Role of a Candida albicans P1-Type ATPase in Resistance to Copper and Silver Ion Toxicity." *J Bacteriol* Sep: 182(17):4,899–905, 2000.

6. Cervantes, C. and F. Guiterrez-Corona. "Copper Resistance Mechanisms in Bacteria and Fungi." *FEMS Microbiol. Rev.* Jun: 14(2):121–37, 1994.

7. Abad, F.X. et al. "Disinfection of Human Enteric Viruses in Water by Copper and Silver in Combination with Low Levels of Chlorine." *Appl. Environ. Microbiol.* Jul: 60(7):2,377–83. 1994.

8. Bruins, M.R., S. Kapil and F.W. Oehme. "Microbial Resistance to Metals in the Environment." *Ecotoxicol Environ Saf* Mar: 45(3):198–207, 2000.

9. Silver, S. and L.T. Phung. "Bacterial Heavy Metal Resistance: New Surprises." *Annu. Rev. Microbiol.* 50:753–89, 1996.

10. Rohr, U. et al. "Four Years of Experience with Silver-Copper Ionization for Control of Legionella in a German University Hospital Hot Water Plumbing System." *Clin Infect Dis.* Dec: 29(6):1,507–11, 1999.