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Efficacy of Copper-Silver Ionization in Controlling Biofilm- and Plankton-Associated Waterborne Pathogens[▽]

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The study was to determine the efficacy of copper-silver ionization against the formation of *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Acinetobacter baumannii* in biofilms and planktonic phases. At concentrations below the EPA limits, ionization has potential to control the three waterborne pathogens, in addition to *Legionella*, in hospital water systems for nosocomial infection control.

Pseudomonas aeruginosa, *Stenotrophomonas maltophilia*, and *Acinetobacter baumannii* are the waterborne pathogens commonly found in chlorinated potable water and linked to nosocomial infections (2, 6, 12, 15, 20–22). These pathogens exist in both free-flowing planktonic cells and biofilm-associated sessile cells adhering to pipe inner surfaces (14, 23). Pathogens persisting in biofilms are much more resistant to disinfectants than planktonic cells of the same isolate (5, 16, 19). The control of pathogens in biofilms is a challenge to health care facilities for prevention of nosocomial infections.

Copper-silver ionization systems have emerged as a long-term disinfection method for *Legionella* in hospital water systems (4, 10, 13, 17, 18). Copper and silver ions have demonstrated *in vitro* efficacy against the waterborne pathogens (9). However, the efficacy against biofilm-associated pathogens has not yet been investigated. Thus, the objective is to evaluate copper and silver ions as a disinfection method against *P. aeruginosa*, *S. maltophilia*, and *A. baumannii* in a model plumbing system that simulates water distribution systems. Our finding may determine if this ionization method can be applied for control of waterborne pathogen colonization in hospital water systems.

Environmental isolates of *P. aeruginosa*, *S. maltophilia*, and *A. baumannii* were selected and prepared as previously described (9). Four liters of bacteria suspension was made to achieve the initial concentration of 3×10^6 CFU/ml for each experiment. The inoculum solution consisted of 4 liters of the bacterial suspension (3×10^6 CFU/ml), 400 liters of dechlorinated tap water, and 1 liter of sterile nutrient supplement solution (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter). The total volume was 405 liters.

A model plumbing system was designed as a partially open system, consisting of four transparent PVC biofilm sampling pipes (Fig. 1). Each experiment was divided into two stages at room temperature: a 14-day inoculation period followed by a 120-hour disinfection. The four-loop system was first inoculated with 405 liters of inoculum solution recirculating through all four loops simultaneously for 14 days at a flow rate of 10 liters/min measured by the flow meter. During the disinfection period, inoculum solutions and disinfectant solution were added into the four individual loops (approximately 100 liters in each loop) and the circulation within each loop was maintained using individual pumps. A 72-h ion maintenance period was selected because we found regrowth of pathogens in both biofilms and planktonic phases within 24 h in prior experiments when the disinfectants were added only at the beginning of the experiment. To overcome this regrowth and better simulate the conditions in the field, we maintained the ion concentrations for the first 72 h at every sampling point and supplied disinfectants, if needed.

The copper-silver ions were generated by a commercial ionization system (Liquidator S50; LiquiTech, Inc., Lombard, IL) at concentrations of Cu/Ag targeted at 0.8/0.08, 0.4/0.04, and 0.2/0.02 mg/liter (EPA limits: Cu, 1.3 mg/liter; Ag, 0.1 mg/liter). The ion concentrations were confirmed by an inductively coupled plasma (PerkinElmer, Waltham, MA). Biofilms and water samples were collected at 0, 3, 6, 12, 24, 48, 72, 96, and 120 h. Biofilm samples were taken by swabbing the inner surface of a premeasured section of the sampling pipe using a sterile swab. The swab was vortexed for 1 min in 2 ml sterilized deionized water with 20 μl neutralizer before plating. A 10-ml planktonic sample was collected from each loop, diluted, and plated onto MacConkey's culture medium for enumeration. Each disinfection experiment for an individual pathogen was conducted at least twice for consistency. SPSS v17.0 software was used for calculation of the 95% confidence interval from the mean value (in logarithms) of each data point.

During the first 72 h of the experiment, when the Cu/Ag concentrations were maintained as described previously, all Cu/Ag concentrations tested (0.2/0.02 to 0.8/0.08 mg/liter) achieved complete inactivation of biofilm-associated *P. aeruginosa* within the first 24 h (Fig. 2a). *P. aeruginosa* concentrations in both biofilms and planktonic samples reached the baseline level after the 72-h ion maintenance period (Fig. 2a and b). This result suggests that maintaining ion concentrations is successful in controlling *P. aeruginosa*. Cu/Ag concentrations tested (0.2/0.02 to 0.8/0.08 mg/liter) achieved complete inactivation of biofilms (3-log reduction) and plankton-associated (>6-log reduction) *S. maltophilia* in 48 h (Fig. 2c and d). Higher Cu/Ag concentrations tested (0.4/0.04 and 0.8/0.08 mg/liter) maintained the reduction even after the 72-h ion main-

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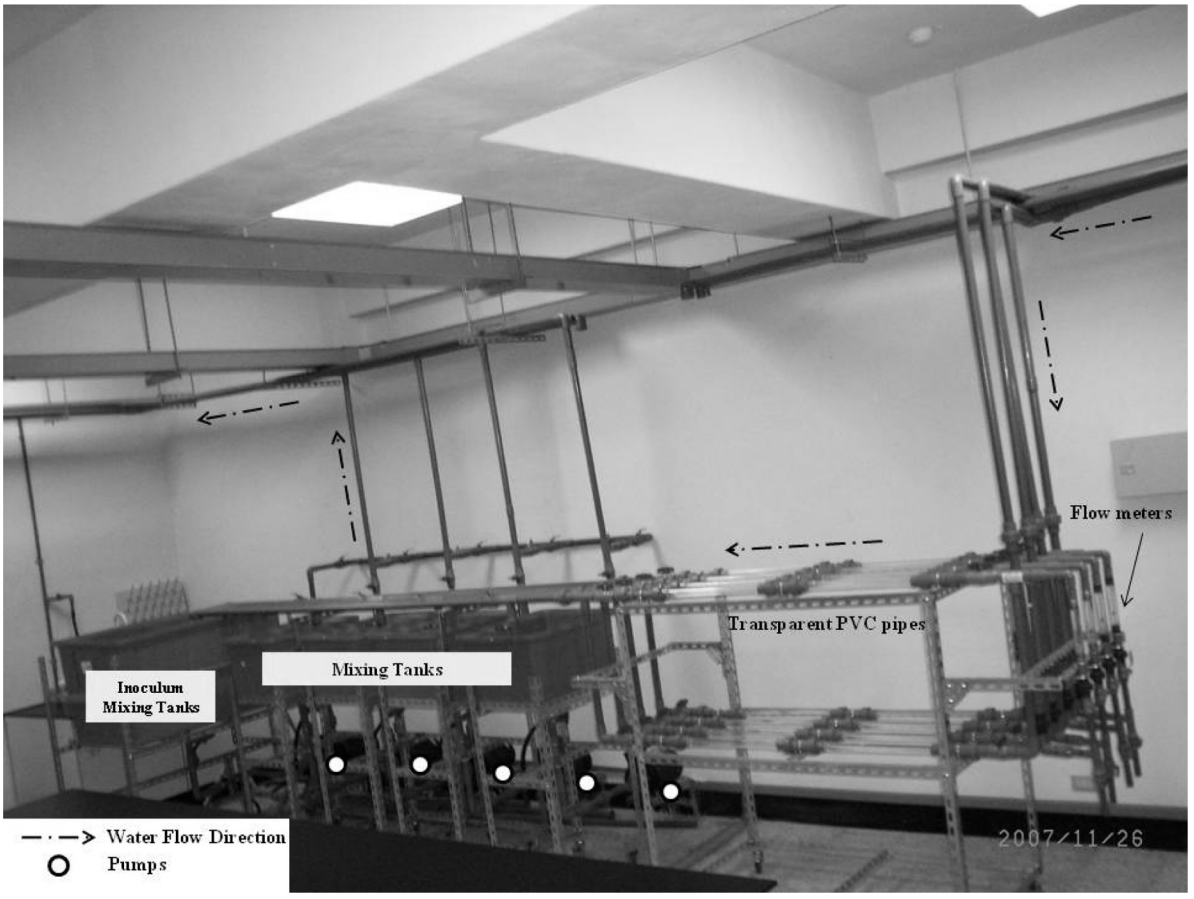

FIG. 1. Model plumbing system.

tenance period, unlike the result with *P. aeruginosa*. The same Cu/Ag concentrations tested achieved 99.9% killing of biofilm-associated *A. baumannii* in 12 h (Fig. 2e). Only Cu/Ag concentrations of 0.8/0.08 mg/liter maintained complete inactivation in the first 72 h. Cu/Ag concentrations of 0.4/0.04 and 0.8/0.08 mg/liter achieved complete inactivation of plankton-associated *A. baumannii* in the first 72 h (Fig. 2f). Less than 1 log regrowth was observed with both biofilms and planktonic samples for *A. baumannii*, unlike the finding for *P. aeruginosa*. *S. maltophilia* appears to be more susceptible to copper and silver ions than *P. aeruginosa* and *A. baumannii*.

The persistence of waterborne pathogens in the hospital water supply system was responsible for hospital-acquired infections. World Health Organization guidelines recommend that water must not be contaminated by waterborne pathogens in the health care setting during storage, distribution, or handling (1). Disinfecting the water system, targeting these pathogens, can be an option for prevention of waterborne pathogen-related infections.

Our results show that copper-silver ionization is effective in controlling biofilms and plankton-associated waterborne pathogens. Although copper and silver ions were added at the appropriate concentrations initially, the regrowth of the test organisms was observed as described previously. This regrowth may be due to the fact that these metallic ions are attached to the test organisms, remain attached throughout the experiment, and have no further killing effect on other organisms (11). This is indicated by the decrease of ion concentrations in the planktonic phase during the first 72 h of each experiment (data not shown). Thus, it is important to maintain proper ion concentrations when applying this method to water systems. In addition, there are measurable decreases in the control populations (i.e., no disinfectants) of plankton-associated *P. aeruginosa* and *S. maltophilia*. We are unable to provide an explanation to describe this observation. It may be because *P. aeruginosa* and *S. maltophilia* are more susceptible to the man-made model plumbing system than *A. baumannii*.

The biofilms and planktonic populations of *P. aeruginosa*, *S. maltophilia*, and *A. baumannii* populations in this study are only 14 days old, much younger than those persisting in the real water distribution system, which may be more resistant to disinfectants. A prospective surveillance should be conducted to validate the efficacy of this procedure in real hospital water systems before it is widely recommended. In addition, copper-silver ionization is a new application to drinking water treatment for *Legionella* and other waterborne pathogens. Registration of copper-silver ionization in drinking water may be required (8) because of adverse effects on human health (3, 7). Currently, ionization manufacturers may continue to offer the technology before the grace period ends.

In summary, copper-silver ionization is efficacious for control of biofilms and plankton-associated waterborne pathogens

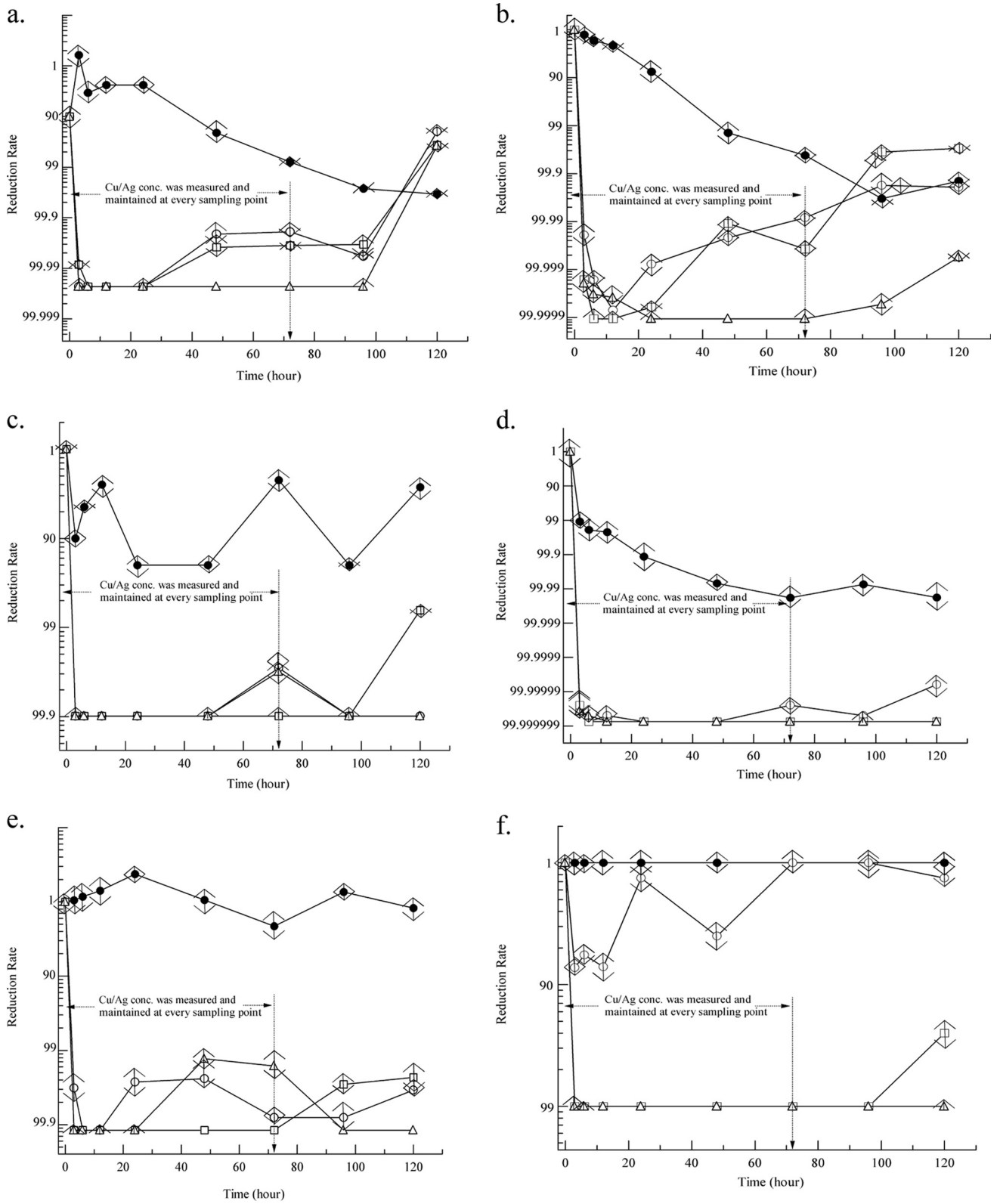

FIG. 2. Efficacy of copper and silver ions in waterborne-pathogen inactivation. ●, control; ○, 0.2/0.02 mg/liter; □, 0.4/0.04 mg/liter; △, 0.8/0.08 mg/liter (Cu/Ag). ↕ indicates 95% confidence interval. (a) Cu/Ag ions achieved more than 99.99% reduction of biofilm-associated *P. aeruginosa* within 24 h. (b) Cu/Ag ions achieved more than 99.999% reduction of plankton-associated *P. aeruginosa* within 12 h. (c) Cu/Ag ions achieved more than 99.9% reduction of biofilm-associated *S. maltophilia* within 48 h. (d) Cu/Ag ions achieved more than 99.99999% reduction of plankton-associated *S. maltophilia* within 72 h. (e) Cu/Ag ions achieved more than 99.9% reduction of biofilm-associated *A. baumannii* within 12 h. (f) Cu/Ag ion concentrations at 0.4/0.04 and 0.8/0.08 mg/liter achieved more than 99% reduction of plankton-associated *A. baumannii* within 100 h.

in a model plumbing system. Copper-silver ionization may be capable of controlling waterborne pathogens, in addition to *Legionella*, in the hospital water distribution system.

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